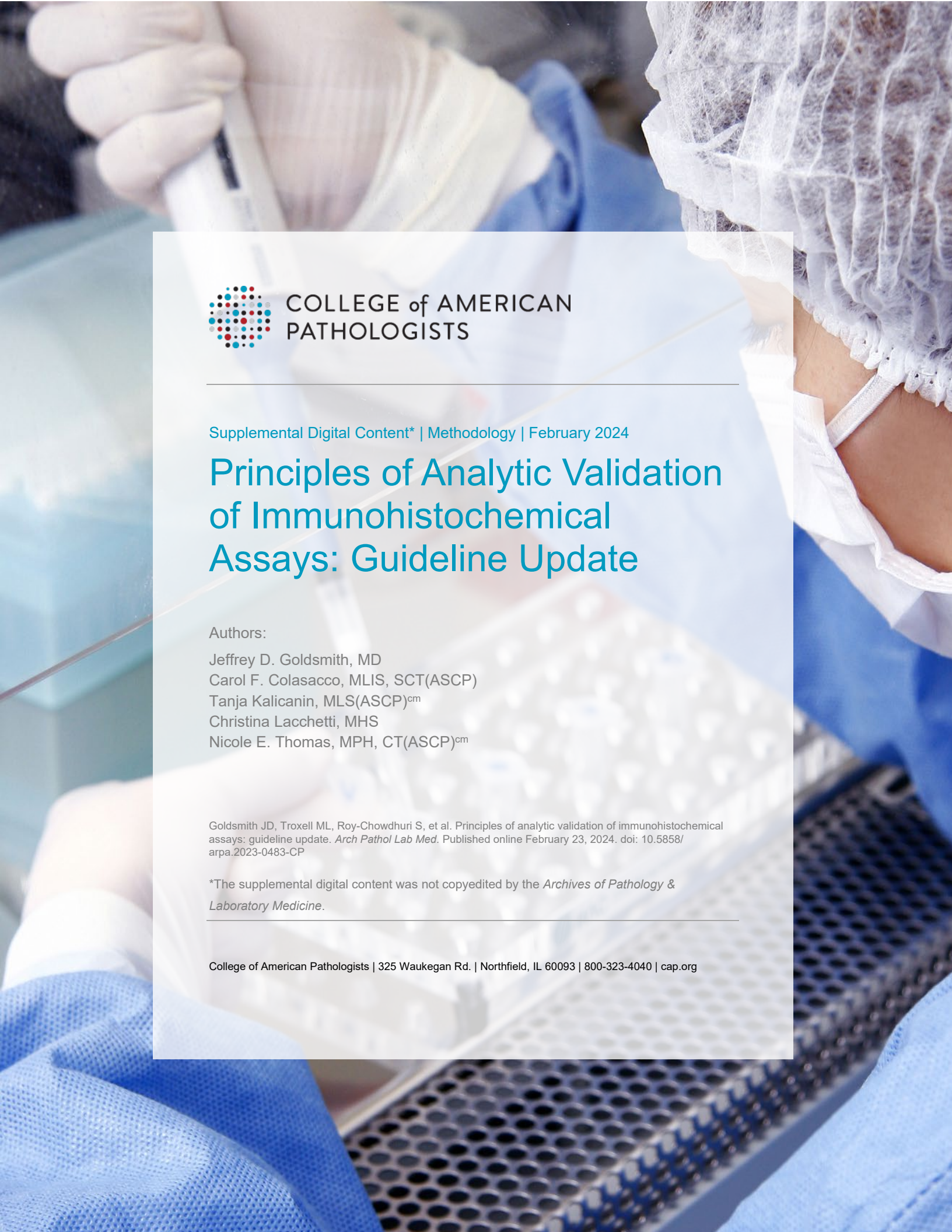

COLLEGE of AMERICAN PATHOLOGISTS

Supplemental Digital Content* | Methodology | February 2024

Principles of Analytic Validation of Immunohistochemical Assays: Guideline Update

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GUIDELINE DEVELOPMENT METHODS

Panel Composition

The College of American Pathologists (CAP) convened an expert and advisory panel (EP/AP) consisting of members with experience and expertise in analytic validation to update the 2014 Principles of Analytic Validation of Immunochemical Assays guideline.[1] Members included practicing pathologists, histotechnologists, and a guideline methodologist. Members were selected to represent diverse laboratory environments and geographic locations to assure that multiple perspectives would be represented. The roles of each panel are described in the Evidence-based Guideline Development Methodology Manual (Methodology Manual).

The EP met via teleconference and one in-person meeting using the Delphi method to come to agreements about scope and recommendations. Work was also conducted via email communication.

Conflict of Interest (COI) Policy

In accordance with the CAP COI policy, members of the expert or advisory panel disclosed all financial interests from 24 months prior to appointment through the development of the guideline, as well as any future relationships planned in the 12 months post-publication. Complete disclosures of the expert panel members are listed in the Appendix. A detailed description of the COI policy is available in the online Methodology Manual.

The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist.

Project Scope and Outcomes of Interest

The EP approved the following key questions (KQs) for the systematic evidence review:

1. For the initial validation of an assay used clinically, what is the minimum overall analytic accuracy?
2. What is the minimum number of positive and negative cases that need to be tested to analytically validate immunohistochemical nonpredictive marker assays, United States Food and Drug Administration (FDA) approved/cleared predictive marker assays (including companion/complementary diagnostics), and laboratory developed predictive marker assays, for their intended use?
3. What parameters should be specified for the tissues used in the validation set?
   a. What tissue/tumor types are appropriate for inclusion in a validation set?
4. How do decalcification and non-formalin fixation methods (including those utilized on cytology specimens) influence analytic validation?
5. What conditions require assay revalidation?

For each key question, the panel identified the Patient/Population, Intervention, Comparator, and Outcomes (PICO) to frame the work. According to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, it is important for clinical guideline panels to review a comprehensive list of outcomes.[2] The key questions and PICO are included in Supplemental Table 2.

Principles of Analytic Validation of Immunohistochemical Assays: Guideline Update
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Search and Selection

Detailed literature searches were constructed using controlled vocabulary and keywords for concepts derived from the PICO elements defined at the onset of the project based upon the key questions. These concepts were: 1) immunohistochemistry, 2) preanalytic factors, and 3) validation. Additional searches to supplement the database searches to identify guidelines and unindexed (grey) literature were completed in Guidelines International Network, [3] ECRI Guidelines Trust, [4] Trip search engine, [5] University of York Centre for Reviews and Dissemination, [6] and relevant US and international organizational websites using the Canadian Agency for Drugs and Technologies in Health (CADTH) Grey Matters document. [7] All search results were deduplicated using reference management software following published methods. [8] Search strategies were reviewed by a second medical librarian. The literature search strategies and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram are included as Supplemental Figures 1 and 2.

Selection at all levels was based on the predetermined inclusion/exclusion criteria, which are detailed in the manuscript.

Systematic Evidence Review (SER)

The objective of the systematic evidence review was to identify articles that would answer the key questions. If of sufficient quality, findings from this review would provide an evidence-base to support the recommendations of the guideline. Each level of the systematic review (title-abstract screening, full-text review, and data extraction) was performed in duplicate by two members of the EP using the systematic review database software, DistillerSR (Evidence Partners Inc., Ottawa, Canada). Conflicts were adjudicated by the chair or methodologist. For data extraction, data elements from an included article/document were extracted by one reviewer into standard data formats and tables developed using DistillerSR; a second reviewer confirmed accuracy and completeness. Any discrepancies in data extraction were resolved by discussion between the chair and the methodologist. A bibliographic database was established in EndNote (Thomson Reuters, Carlsbad, CA) to track all literature identified and reviewed.

Assessing Quality and Risk of Bias

An assessment of the quality of the evidence was performed for all retained studies following application of the inclusion and exclusion criteria. Using this method, studies deemed to be of low quality would not be excluded from the systematic review but would be retained, and their methodological strengths and weaknesses discussed where relevant. To define an overall risk of bias rating for each included study, validated study-type specific tools were used to assess the risk of bias, plus additional important quality features were extracted. Specific details for each study type are outlined below.

Systematic Reviews (SRs) and Meta-analyses questions were assessed as per the Assessing the Methodological Quality of Systematic Reviews (AMSTAR) 8 tool. [9]

Non-randomized studies

- The following domains were assessed using the Risk of Bias in Non-Randomized Studies – of Intervention (ROBINS-I) tool[10] using low risk, moderate risk, serious risk, critical risk, or unclear:
    1. Confounding
    2. Patient selection (selection bias)
    3. Intervention classification (performance bias)

4. Deviation from intended intervention (performance bias)
5. Missing data (reporting bias)
6. Outcome measurements (detection bias)
7. Selection of reported outcomes (detection bias)

Diagnostic studies were assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool. [11]

Assessing the Certainty of Evidence and Strength of Recommendations

Development of recommendations required that the panel review the identified evidence and make a series of key judgments:

1. What are the significant findings related to each KQ or outcome? Determine any regulatory requirements and/or evidence that support a specific action.
2. What is the overall certainty of evidence supporting each KQ or outcome? Certainty of evidence is graded as High, Moderate, Low, and Very Low, based on published criteria (Supplemental Table 3). Certainty of evidence is a key element in determining the strength of a recommendation. Supplemental Tables 4 – 11 includes the detailed risk of bias assessment and overall certainty of evidence that supports the KQs and outcomes.
3. What is the strength of each recommendation? The strength of recommendations is designated as Strong or Conditional (refer to Table 1 in the manuscript). There are many methods for determining the strength of a recommendation based on the certainty of evidence and the magnitude of net benefit or harm. According to the GRADE approach, the strength of a recommendation demonstrates the extent to which an EP is "confident that the desirable effects of an intervention outweigh undesirable effects". [12] For each statement, the panel rated each GRADE evidence-to-decision framework (EtD) domain. [13] With a strong recommendation designation, the EP judgements will mostly be favoring the right or left of the framework and indicate high confidence that the desirable effects of the guidance statement outweigh the undesirable effects. With a conditional recommendation, the EP judgements will be more towards the center of the framework or with a dispersed pattern indicating lower confidence. Statements not supported by evidence (ie, evidence was missing or insufficient to permit a conclusion to be reached) and made based on consensus expert opinion were included as Good Practice Statements and did not undergo the EtD review. [14]

Evidence-to-Decision Framework (EtD) Domains

Problem Priority	• Is the problem a priority and a recommendation is needed to address it? • Are there consequences that are serious if the problem is not addressed?
Benefits and Harms	• Are the desirable anticipated effects large? • Are the undesirable anticipated effects small? • Are the desirable effects large relative to undesirable effects?

Values and Preferences of Stakeholders	• Is there certainty of how stakeholders (patients, clinicians) value the outcomes? • Is there variability on how patients and clinicians value the outcomes? • Will there be different decisions from key stakeholders because of the different values placed on the outcomes?
Resources Required	• If the Recommendation is made, how large are the resource requirements?
Health Equity	• Are there groups or settings that might be disadvantaged in relation to the Recommendation being considered? • Are there different baseline conditions across groups or settings that affect the absolute effectiveness of the Recommendation or the importance of the problem for disadvantaged groups or settings? • Are there important considerations that should be made when implementing the Recommendation in order to ensure that inequities are reduced, if possible, and that they are not increased?
Feasibility	• Is the option (or recommendation) feasible to implement? • Is the Recommendation sustainable? Are there important barriers that are likely to limit the feasibility of implementing the Recommendation? If yes, do these barriers require consideration when implementing the Recommendation?
Acceptability	• Is the option acceptable to key stakeholders? • Are there key stakeholders that would not accept the distribution of the benefits, harms or costs? • Are there key stakeholders that would not accept the costs or undesirable effects in the short term for desirable effects (benefits) in the future?

Data derived from Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) Working Group Materials. [2]

Open Comment Period and Organizational Review

An open comment period was held from August 4-25, 2021, on the CAP web site (www.cap.org). Recommendations and good practice statements, demographic questions, and questions to assess feasibility were posted for peer review. An announcement was sent to the following societies/organizations deemed as stakeholders:

- Association for Molecular Pathology (AMP)
- American Society for Clinical Pathology (ASCP)
- Association of Directors of Anatomic and Surgical Pathology (ADASP)
- Association of Pathology Chairs (APC)

- United States & Canadian Academy of Pathology (USCAP)
- Canadian Association of Pathologists (CAP-APC)
- CAP
- American Society for Clinical Oncology (ASCO)
- American Society of Cytopathology (ASC)
- International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
- Nordic IHC Quality Control (NordicQC) Program
- Canadian Pathology Quality Assurance (CPQA) – Assurance qualite candienne en pathologie
- National Society for Histotechnology (NSH)
- Society to Improve Diagnoses in Medicine (SIDM)
- Papanicolaou Society of Cytopathology (PSC)
- International Academy of Pathology (IAP)
- UK NEQAS
- Society for Immunotherapy of Cancer (SITC)
- American Cancer Society (ACS)
- Kaiser Permanente/Kaiser Family Foundation (KP/KFF)
- Centers for Medicare & Medicaid Services (CMS)
- Centers for Disease Control and Prevention (CDC)
- National Institutes of Health, Division of Cancer Treatment and Diagnosis (DCTD)
- United Kingdom National External Quality Assessment Service (UK NEQAS)
- China Food and Drug Administration (CFDA)
- European Medicines Agency (EMA)
- National Institute for Health and Care Excellence (NICE)
- US Food and Drug Administration (FDA)
- Veteran’s Affairs (VA) and Department of Defense (DOD)

For each guideline statement, participants were asked to select among the following responses: “Agree as written”, “Agree with suggested modifications”, “Disagree”, and “Neutral”. The website received more than 350 written comments. Seven draft statements achieved more than 90% agreement, 6 statements received between 80%-90%, and 1 draft statement received below the 80% agreement. Volunteer EP members were assigned draft recommendation statements for which members reviewed the comments and provide suggestions to the entire panel to keep original draft language, edit with minor changes for clarity, or edit with major changes. After consideration of the comments, a total of three recommendations and 12 good practice statements were included in the guideline – most undergoing minor editing for clarity, but one statement originally presented during the comment period was separated into two different statements. Resolution of all changes was obtained by majority consensus of the panel using nominal group technique (discussion during teleconference webinars, email discussion, and multiple edited recommendations) amongst the panel members. The final recommendations and good practice statements were approved by the EP with a formal vote.

Organizational review was instituted to review and approve the guideline. An independent review panel (IRP) representing the Council on Scientific Affairs was assembled to review and approve the guideline for the CAP. The IRP was masked to the expert panel and vetted through the COI process.

Dissemination Plans

The CAP plans to host a Principles of Analytic Validation of Immunohistochemistry (IHC) Assays guideline update resource webpage which will include a link to the manuscript and supplement; a summary of the recommendations, a teaching PowerPoint (Microsoft Corporation, Redmond, WA), a frequently asked question (FAQ) document, and an infographic along with other additional tools such as webinar recordings as applicable. The guideline will be promoted and presented at various society meetings.

Recommendation Statements

Statement 2. Strong Recommendation – 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.

This statement maintains the 2014 guideline recommendation. As such, laboratories are familiar with and have already been using the 90% concordance benchmark. It is of great benefit to have an achievable and feasible concordance standard. While greater than 90% concordance is preferable, especially for predictive markers, the panel believes that less than 90% is unacceptable and likely indicates inferiority of the new assay. The panel believes that this recommendation balances patient safety and feasibility. The panel acknowledges that there is a margin of acceptable error (between 90-100% concordance) and the fact that analytic validation of new assays, though necessary, is a time consuming and sometimes costly task.

Statement 6. Strong Recommendation – For initial analytic validation of laboratory developed assays and verification of FDA-approved/cleared predictive immunohistochemical assays with distinct scoring schemes (eg, human epidermal growth receptor 2 [HER2] and programmed death receptor-1 [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.

This is a new statement based on the evolution of clinical immunochemistry that recently recognized the distinction between IHC readout and interpretation. Addressing analytic validation of assays with distinct scoring systems was considered to be a high priority to the expert panel. Given that the readout is a critical portion of the analytical phase of testing, the expert panel believes that separate validation of each assay-scoring system combination would result in large benefits. Despite the fact that the expert panel believes that this additional activity would pose potentially substantial additional burden on laboratories that run these sorts of assays, we believe that this additional step will help to assure that the correct patient results will be reported on these critical predictive marker assays whose results will be used to make important treatment decisions.

Statement 9. Conditional Recommendation – 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.

***Note*: Such cytologic specimens include (but are not necessarily limited to):**

- **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 post-fixed in formalin**

Like the previous statement, this is a new recommendation. Creation of more definitive recommendations for analytic validation of IHC assays performed on cytologic specimens was a priority for the expert panel based on feedback received from the original guideline. The panel elected to include this recommendation based chiefly on the fact that new literature has emerged that generally shows that immunohistochemistry performed on non-formalin fixed and/or alternatively processed cytologic specimens often has decreased sensitivity compared to IHC performed on formalin-fixed, paraffin-embedded tissue (FFPE). The panel recognizes that this recommendation will impose significant additional burdens on laboratories that perform IHC on cytology specimens that are processed and fixed differently than their FFPE histology counterparts. However, the systematic review showed that this new recommendation is supported by the literature and would provide laboratories with additional recommendations that would improve assay quality on cytology specimens.

Supplemental Table 1. Glossary of Guideline Terms

Term	Definition for the purpose of this guideline
Accuracy	The degree of correctness or true values of a given laboratory result comparing to a gold standard. [15] Note: As IHC assays lack gold standards, this guideline will use concordance.
Analyte specific reagent	Antibodies, both polyclonal and monoclonal, or similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical or ligand in biological specimens. [16, 17]
Assay, IHC	The technical components of the immunohistochemical testing process, exclusive of interpretation or reporting. [18]
Biomarker	A physiological analyte that is objectively measured and evaluated as an indicator of normal biological and pathogenic processes or expected pharmacological responses to a specified therapeutic intervention. [19]
Companion diagnostic assay	An in vitro diagnostic device that provides information that is essential for the safe and effective use of a corresponding therapeutic product. For FDA-approved therapeutics, the use of a companion diagnostic is typically stipulated in the labelling of both. [20, 21]
Complementary diagnostic assay	Assays that identify a biomarker-defined subset of patients that typically respond to a drug and aid risk/benefit assessments for individual patients, but that are not pre-requisites for receiving the drug. [20]
Concordance, overall	Also known as percent agreement, is a measure used for comparison of the results of the new test to those obtained using a non-gold standard reference assay (or an “imperfect standard”). [1]
Concordance, negative	The proportion of “negative” samples in which the index test is negative. [1]
Concordance, positive	The proportion of “positive” samples in which the index test is positive. [1]
False negative	A negative test result for a patient or specimen that is known or subsequently proved positive for the condition or constituent in question. [18]
False positive	A positive test result for a patient or specimen that is known or subsequently proved to be negative for the condition or constituent in question. [18]

FDA-approved assay	Assays that are approved for marketing under the FDA premarket approval process for new devices, requiring demonstration of safety and effectiveness of Class III devices. [16, 22]
FDA-cleared assay	Assays that are cleared for marketing under the FDA 510(k) review, and such clearance is reserved for devices that are substantially equivalent to those already on the market for which there is a predicate IHC device. [16] Note: Less stringent than FDA premarket approval.
FDA Class I IHC: Diagnostic markers, Nonpredictive markers	IHCs being used as adjuncts to conventional histopathologic diagnostic examination and with readily available internal and external control materials. These IHC results are evaluated and incorporated into the diagnostic interpretation by the pathologist. [1, 17]
FDA Class II IHC	IHCs intended for the detection and/or measurement of certain target analytes by immunological techniques in order to provide prognostic and predictive data that are not directly confirmed by routine histopathologic internal and external control specimens. These IHCs provide the pathologist with diagnostic information that is ordinarily reported as independent diagnostic information to the ordering clinician. [1, 17]
FDA Class III IHC	IHCs that do not meet the criteria for class I or II. Manufacturers of these IHCs must submit valid scientific evidence to support the new intended uses (FDA approval/clearance). IHCs identifying new, clinically significant target analytes in tissue specimens that cannot be confirmed by conventional histopathologic examination. [17]
Fit for purpose	An assay that has been successfully validated for the intended use, combining both laboratory and clinical definitions, and factoring in the disease, diagnostic assay, and where applicable, the drug. [23, 24]
Laboratory developed test or assay (LDT)	A type of in vitro diagnostic test that is designed, manufactured and used within a single laboratory according to the laboratory's own procedures. Note: LDTs may derive from any of the following scenarios: (1) A testing laboratory develops and validates an IHC assay from first principles using separately purchased, commercially available components (aka "de novo LDT"); (2) A testing laboratory adds/subtracts/modifies any manufacturer specified preanalytical, analytical, or postanalytical component/aspect of a commercially available, regulatory agency–approved IHC assay/in vitro diagnostic device, or uses it for a purpose other than intended by the manufacturer. [15, 19]
Laboratory Modified Test	FDA-cleared/approved assays with modification. Modified assays are considered LDTs. Note: Relevant modifications include but are not limited to changes to the manufacturer's supplied ingredients, instrumentation or procedure, as well as change of specimen type, specimen preanalytics, or stated purpose of the test, its approved test population, or any claims related to interpretation of the results. [16]
Optimization	The process by which the laboratory serially tests and modifies components of the assay to maximize the signal to noise ratio prior to validating the assay for specific clinical purposes.
Predictive value, negative (NPV)	Probability that a person who has tested negative does not have the biomarker present. [25]

Predictive value, positive (PPV)	Probability that a person who has tested positive actually has the biomarker present. [25]
Predictive marker	Biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from a (targeted) therapy. [21, 26] Note: Predicts responsiveness to a specific treatment among cases of the same diagnosis; independent of other histopathologic findings.
Prognostic marker	Biomarker used to identify likelihood of a clinical event, disease recurrence, or progression in patients, regardless of the treatment. [18, 19, 21]
Purpose	Intended use at the time the test was developed. [24] Note: See Fit-for-Purpose.
Readout	The determination of the intensity, extent, quality, and cellular localization of immunohistochemical signal.
Repeatability	Within run reproducibility. [16]
Reproducibility	Extent of agreement among results obtained by replicate testing of specimen sets between laboratories, testing platforms or readers. [1, 16] Note: Similar to precision, for qualitative testing.
Revalidation	A process to assess a previously validated test's accuracy and reliability in detecting the marker of interest when there has been a change in test conditions, such as methods, reagents, instrumentation, fixation, specimen types, purpose. [15]
Robustness	Assay reproducibility in the face of changes in various test conditions, such as relevant range of preanalytical conditions, instruments, operators. [25]
Sensitivity, diagnostic	The proportion of those with the target condition (as defined by a reference standard) who test positive with a candidate test. [19] Note: As most IHC assays lack a gold standard, this guideline uses concordance.
Sensitivity, analytical	The ability to obtain positive results in concordance with positive results obtained by the reference method. [16]
Specificity, analytical	The ability to obtain negative results in concordance with negative results obtained by the reference method. [16]
Specificity, diagnostic	The proportion of those without the target condition (as defined by a reference standard) who test negative with a candidate assay. [19] Note: As most IHC assays lack a gold standard, this guideline uses concordance.
Validation	A process to establish that the performance of a test, tool, or instrument is acceptable for its intended purpose. Validation establishes the performance characteristics of an assay as well as the assay limitations. [19]
Validation, analytical (technical)	The process used to confirm with objective evidence that a laboratory-developed or modified FDA-cleared/approved test method or instrument system delivers reliable results for the intended application. [21, 26]
Validation, indirect clinical	The process used to determine that an assay delivers reliable results as compared to a designated previously *clinically* validated reference assay. The comparator assay may or may not be FDA-approved, but it must be qualified/validated in a prospective clinical trial, with established link to clinical outcome. [19]
Verification, analytical	A process by which a laboratory determines that an unmodified FDA-cleared/ approved test performs according to the specifications set forth by the manufacturer when used as directed. [26]

Abbreviations: FDA, Food and Drug Administration; IHC, immunohistochemistry

Supplemental Table 2. Key Questions and PICO Elements

KQ1 For the initial validation of an assay used clinically, what is the minimum overall analytic accuracy?			
Population	**Intervention**	**Comparator**	**Outcomes**
All IHC assays used clinically	Either <90% or >90%	90%	Diagnostic accuracy
KQ2 What is the minimum number of positive and negative cases that need to be tested to analytically validate immunohistochemical nonpredictive marker assays, FDA approved/cleared predictive marker assays (including companion/complementary diagnostics), and laboratory developed predictive marker assays, for their intended use?			
Population	**Intervention**	**Comparator**	**Outcomes**
Nonpredictive marker IHC assays being validated for clinical use	At least 20 cases (10 positive and 10 negative)	Any other number of cases	Diagnostic accuracy
FDA approved/cleared predictive marker IHC assays being validated for clinical use	40 cases (20 positive and 20 negative)	Any other number of cases	Diagnostic accuracy
Laboratory-developed predictive marker assays being validated for clinical use	40 cases (20 positive and 20 negative)	Any other number of cases	Diagnostic accuracy
KQ3 What parameters should be specified for the tissues used in the validation set? a. What tissue/tumor types are appropriate for inclusion in a validation set?			
No PICO – addresses fit-for-purpose			
KQ4 How do decalcification and non-formalin fixation methods (including those utilized on cytology specimens) influence analytic validation?			
Population	**Intervention**	**Comparator**	**Outcomes**
IHC assays performed on decalcified tissue or non-formalin fixed tissue or cells being validated for clinical use	Analytic accuracy on decalcified or non-formalin fixed tissue	Analytic accuracy on non-decalcified formalin fixed tissue	Diagnostic accuracy
KQ5 What conditions require assay revalidation?			
Change in antibody lot			
Population	**Intervention**	**Comparator**	**Outcomes**
IHC assays	New antibody lot (no change in clone)	Old antibody lot	Minimum acceptable concordance
Change in antibody dilution, antibody vendor (same clone), primary antibody incubation, antigen retrieval times.			
Population	**Intervention**	**Comparator**	**Outcomes**

IHC assays	New primary antibody dilution, antibody vendor, etc.	Old primary antibody dilution, antibody vendor, etc.	Diagnostic accuracy
Antigen retrieval method, antigen detection system, tissue processing or testing equipment, environmental conditions of testing, laboratory water supply			
Population	**Intervention**	**Comparator**	**Outcomes**
IHC assays	New antibody clone	Old antibody clone	Diagnostic accuracy
Change in antibody clone			
Population	**Intervention**	**Comparator**	**Outcomes**
IHC assays	New antibody clone	Old antibody clone	Diagnostic accuracy

Abbreviations: FDA, Food and Drug Administration; IHC, immunohistochemistry; PICO, population, intervention, comparator, outcomes; KQ, key question

Supplemental Table 3: Certainty of Evidence

Designation	Description
High	There is high confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect. Included studies will be of high or intermediate quality.
Moderate	There is moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate. Included studies will be of intermediate or low quality.
Low	There is limited confidence in the estimate of effect. The true effect may be substantially different from the estimate of the effect. Included studies will be of low quality.
Very Low	There is very little confidence in the estimate of effect. The true effect is likely to be substantially different from the estimate of effect. Any estimate of effect is very uncertain. Included studies will be of low or very low quality.

Data derived from Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group Materials. [2]

Supplemental Table 4. Risk of Bias Assessment of Included Systematic Reviews

AMSTAR	Girolami et al, 2022[27]	Voidazan et al, 2022[28]
A priori design	√	√
Duplicate study selection & data extraction	√	√
Comprehensive lit search performed	√	x
Grey lit used	√	x
List included & excluded studies	x	x
Characteristics of included studies provided	√	√

Quality assessed & documented	√	x
Quality used appropriately for conclusion	√	x
Methods to combine used appropriately	√	√
Publication bias assessed	x	x
COI	√	√
AMSTAR SCORE /11	**9**	5

Abbreviations: AMSTAR, Assessing the Methodological Quality of Systematic Reviews; COI, conflict of interest

Supplemental Table 5. Risk of Bias Assessment of Included Consecutive Series of Patients Studies

Study	ROBINS-I Assessment							
	Confounding	Patient Selection	Intervention classification	Deviations from intended interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall Risk of Bias
Fujimoto et al, 2018[29]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Guo et al, 2018[30]	low	low	low	low	low	low	low	low
Ma et al, 2018[31]	low	low	low	low	low	low	low	low
Fujimoto et al, 2018[32]	moderate	moderate	serious	moderate	moderate	moderate	moderate	serious
Paja Fano et al, 2017[33]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Zhang et al, 2018[34]	moderate	low	low	low	low	low	low	low
Sunshine et al, 2017[35]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Marchetti et al, 2016[36]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate
Zhu et al, 2016[37]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Shan et al, 2015[38]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Shan et al, 2014[39]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Ying et al, 2013[40]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Wang et al, 2018[41]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate
Sener et al, 2017[42]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Hirsch et al, 2017[43]	moderate	serious	moderate	moderate	moderate	serious	moderate	serious
Burel-Vandenbos et al, 2017[44]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Toi et al, 2018[45]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Saito et al, 2018[46]	moderate	moderate	serious	moderate	moderate	serious	moderate	serious
Smith et al, 2018[47]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Noll et al, 2018[48]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Sakane et al, 2018[49]	moderate	moderate	serious	moderate	moderate	serious	moderate	serious

Soo et al, 2018[50]	moderate	moderate	serious	moderate	moderate	serious	moderate	serious
Vlajnic et al, 2018[51]	moderate	low	moderate	moderate	low	moderate	moderate	moderate
Ramteke et al, 2018[52]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Kim et al, 2017[53]	moderate	moderate	serious	moderate	moderate	serious	moderate	serious
Xu et al, 2017[54]	moderate	moderate	serious	moderate	moderate	serious	moderate	serious
Geethamala et al, 2017[55]	moderate	low	serious	moderate	moderate	serious	moderate	serious
Kim et al, 2016[56]	moderate	low	serious	moderate	moderate	low	moderate	serious
Handa et al, 2015[57]	moderate	low	serious	moderate	moderate	moderate	moderate	serious
Shubham et al, 2016[58]	moderate	low	serious	moderate	moderate	moderate	moderate	serious
Nishimura et al, 2016[59]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Ragazzi et al, 2016[60]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Long et al, 2015[61]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate
Jalaly et al, 2015[62]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Zhang et al, 2015[63]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Lee et al, 2015[64]	moderate	low	moderate	moderate	low	low	moderate	moderate
Nishida et al, 2015[65]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Srebotnik et al, 2015[66]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Pearlstein et al, 2014[67]	moderate	moderate	serious	moderate	moderate	low	moderate	serious
Durgapal et al, 2014[68]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Savic et al, 2013[69]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Minca et al, 2013[70]	moderate	moderate	serious	moderate	moderate	moderate	moderate	serious
Mardanpour et al, 2012[71]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Dugas et al, 2019[72]	moderate	low	moderate	moderate	moderate	moderate	moderate	moderate
Gargano et al, 2021[73]	moderate	low	moderate	moderate	moderate	low	moderate	moderate
Ambrosini-Spaltro et al, 2021[74]	moderate	moderate	moderate	moderate	moderate	serious	moderate	serious
Muggilli et al, 2021[75]	moderate	low	moderate	moderate	low	moderate	moderate	moderate
Omar et al, 2021[76]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate
Ulain et al, 2022[77]	moderate	low	moderate	low	moderate	low	moderate	moderate

Ronchi et al, 2022[78]	moderate	low	moderate	low	low	moderate	moderate	moderate
Rohilla et al, 2022[79]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Rashed et al, 2023[80]	moderate	moderate	moderate	moderate	low	moderate	moderate	moderate
Rachagiri et al, 2022[81]	moderate	moderate	moderate	moderate	low	moderate	moderate	moderate
Okuno et al, 2022[82]	moderate	moderate	moderate	moderate	low	moderate	moderate	moderate
Mahajan et al, 2022[83]	moderate	moderate	moderate	moderate	low	moderate	moderate	moderate
Lynggard et al, 2022[84]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Lou et al, 2023[85]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate
Idrees et al, 2022[86]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate
Hjerpe et al, 2023[87]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Han et al, 2022[88]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Garcia et al, 2023[89]	moderate	moderate	moderate	low	low	moderate	moderate	moderate
Frankel et al, 2022[90]	moderate	moderate	moderate	low	moderate	moderate	moderate	moderate
Doonan et al, 2022[91]	moderate	serious	moderate	moderate	low	low	moderate	moderate
Ahmed et al, 2022[92]	moderate	serious	moderate	moderate	low	moderate	moderate	moderate
Ungureanu et al, 2021[93]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Song et al, 2021[94]	moderate	moderate	moderate	moderate	low	low	low	moderate
Mansour et al, 2021[95]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Mallick et al, 2021[96]	moderate	moderate	low	low	moderate	low	low	moderate
Ma et al, 2021[97]	moderate	serious	moderate	moderate	moderate	moderate	moderate	serious
Kumar et al, 2021[98]	moderate	moderate	serious	moderate	moderate	serious	moderate	serious
Fu et al, 2021[99]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Carcea et al, 2021[100]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Bhattacharya et al, 2021[101]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Anand et al, 2021[102]	serious	serious	moderate	moderate	moderate	serious	moderate	serious
Ireka et al, 2019[103]	moderate	moderate	moderate	moderate	moderate	low	moderate	moderate

Koomen et al, 2021[104]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate

Abbreviations: ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention

Supplemental Table 6. Risk of Bias Assessment of Included Randomized Series of Patients Studies

Study	ROBINS-I Assessment							
	Confounding	Patient Selection	Intervention classification	Deviations from intended interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall Risk of Bias
Gaule et al, 2017[105]	moderate	low	serious	serious	moderate	moderate	moderate	serious
Phillips et al, 2018[106]	low	low	low	low	low	low	low	low
Rimm et al, 2017[107]	moderate	moderate	serious	moderate	moderate	moderate	moderate	serious
Ilie et al, 2018[108]	moderate	low	moderate	moderate	low	low	moderate	moderate
Huange et al, 2016[109]	moderate	serious	moderate	moderate	moderate	serious	moderate	serious
Perez et al, 2015[110]	moderate	low	low	low	moderate	low	low	low

Abbreviations: ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention

Supplemental Table 7. Risk of Bias Assessment of Included Non-randomized Series of Patients Studies

Study	ROBINS-I Assessment							
	Confounding	Patient Selection	Intervention classification	Deviations from intended interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall Risk of Bias
Hernandez et al, 2019[111]	low	low	low	low	low	low	low	low
Munari et al, 2019[112]	moderate	serious	moderate	moderate	moderate	moderate	moderate	moderate

Bisschop et al, 2018[113]	low	Low	Low	Low	moderate	Low	Low	moderate
Batenchuk et al, 2018[114]	moderate	moderate	low	moderate	moderate	moderate	moderate	moderate
De Meulenaere et al, 2018[115]	serious	moderate	Serious	moderate	moderate	serious	moderate	serious
Rebelatto et al, 2016[116]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	serious
Xu et al, 2016[117]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Tay et al, 2017[118]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Skov et al, 2017[119]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Thorne-Nuzzo et al, 2017[120]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Berghoff et al, 2017[121]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Lenos et al, 2017[122]	serious	moderate	moderate	moderate	serious	moderate	moderate	serious
Russell-Goldman et al, 2018[123]	serious	moderate	moderate	moderate	moderate	serious	moderate	serious
Woo et al, 2018[124]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Koppel et al, 2018[125]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Watanabe et al, 2018[126]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Hodgson et al, 2018[127]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Bogdanovska-Todorovska et al, 2018[128]	serious	moderate	moderate	moderate	moderate	serious	moderate	serious
Russell-Goldman et al, 2018[129]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Veena et al, 2017[130]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Wang et al, 2015[131]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Lo et al, 2016[132]	serious	moderate	serious	moderate	moderate	serious	moderate	serious
Luttmer et al, 2016[133]	moderate	moderate	moderate	moderate	serious	moderate	moderate	serious
Tanaka et al, 2016[134]	serious	moderate	moderate	moderate	serious	serious	moderate	serious
Acs et al, 2016[135]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious

Sauter et al, 2016[136]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Lee et al, 2016[137]	serious	moderate	moderate	moderate	moderate	moderate	serious	serious
Hoshikawa et al, 2016[138]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Brandler et al, 2015[139]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Li et al, 2015[140]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Savic et al, 2015[141]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Goschzik et al, 2015[142]	serious	moderate	serious	moderate	moderate	serious	moderate	serious
Yadav et al, 2014[143]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Killeen et al, 2014[144]	serious	moderate	serious	moderate	moderate	serious	moderate	serious
Konofas et al, 2013[145]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Capper et al, 2013[146]	serious	moderate	moderate	moderate	moderate	moderate	moderate	serious
Babu et al, 2019[147]	serious	moderate	serious	moderate	moderate	serious	moderate	serious
Sun et al, 2016[148]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Elsharkawy et al, 2014[149]	serious	moderate	moderate	moderate	moderate	moderate	moderate	moderate

Abbreviations: ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention

Supplemental Table 8. Risk of Bias Assessment of Included Observational Descriptive Studies

Study	ROBINS-I Assessment							
	Confounding	Patient Selection	Intervention classification	Deviations from intended interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall Risk of Bias
Lloyd et al, 2019[150]	moderate	moderate	low	moderate	low	low	low	moderate
Ilie et al, 2018[151]	moderate	moderate	low	moderate	low	moderate	moderate	moderate

Villaruz et al, 2019[152]	moderate	moderate	moderate	moderate	serious	moderate	moderate	serious
Hendry et al, 2018[153]	serious	serious	moderate	moderate	moderate	serious	moderate	serious
Tretiakova et al, 2018[154]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Viswanathan et al, 2022[155]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate

Abbreviations: ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention

Supplemental Table 9. Risk of Bias Assessment of Included Studies of Other Designs

Study	ROBINS-I Assessment							
	Confounding	Patient Selection	Intervention classification	Deviations from intended interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall Risk of Bias
Bashover et al, 2019[156]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Capizzi et al, 2018[157]	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Gruver et al, 2014[158]	moderate	moderate	low	moderate	moderate	low	moderate	moderate
Malik et al, 2022[159]	series	series	moderate	moderate	low	moderate	moderate	serious

Abbreviations: ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention

Supplemental Table 10. Risk of Bias Assessment of Included Diagnostic Studies

Study	QUADAS			Overall Risk of Bias
	Are there concerns that the included patients do not match the review question?	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?	
Dvorak et al, 2014[160]	low	low	low	low
Straccia et al, 2019[161]	unclear	unclear	unclear	moderate
Jain et al, 2018[162]	unclear	unclear	unclear	moderate

Jiang et al, 2014[163]	unclear	low	low	moderate

Abbreviations: QUADAS, Quality Assessment of Diagnostic Accuracy Studies

Supplemental Table 11. GRADE Certainty of Evidence Assessment

Outcome	# of Studies	Design	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other	Importance	Certainty of Evidence Grade for Outcome	Overall Certainty of Evidence Grade for Statement
RECOMMENDATION 2										
Concordance	5	4 NRS, 1 CS	Serious	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate
RECOMMENDATION 6										
PD-L1 & HER2										
Concordance	25	11 CS, 7 NRS, 3 observational descriptive, 4 randomly selected series	Serious	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate
Companion Diagnostics										
Concordance	23	13 CS, 7 NRS 1 survey, 1 randomly selected series, 1 diagnostic study	Moderate	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate
RECOMMENDATION 9										

Sen/Spec	64	39 CS, 18 NRS, 2 observational descriptive, 2 diagnostic studies, 2 SR/MA and 1 other study design	Serious	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate
PPV/NPV	41	28 CS, 12 NRS, 1 observational descriptive	Serious	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate
Concordance	34	19 CS, 9 NRS, 1 observational descriptive, 1 randomly selected series of patients, 1 diagnostic study and 3 other study designs	Serious	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate
Dx Acc	14	8 CS, 4 NRS, 1 observational descriptive and 1 other study design	Serious	Not serious	Not serious	Not serious	None	Critical	Moderate	Moderate

Abbreviations: HER2, human epidermal growth receptor 2; PD-L1, programmed death receptor-1; NRS, Non-randomized study; CS, Consecutive series of patients; Dx Acc, Diagnostic accuracy; NPV negative predictive value; PPV, positive predictive value; Sen, sensitivity; Spec, specificity; SR/MA, systematic review/meta-analysis

Supplemental Figure 1: Database Search Strings

Ovid MEDLINE Search String:

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R)

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1 immunohistochemistry/mt, st
2 *immunohistochemistry/
3 (immunohistochem$ or immunocytochem$ or immunoperoxidase or IPX or IHC).ti,kf.
4 (immunohistochem$ or immunocytochem$ or immunoperoxidase or IPX or IHC).ab. /freq=2
5 or/1-3
6 or/1-4
7 immunohistochemistry/
8 6 or 7
9 validation studies/
10 validation studies as topic/
11 *"feasibility studies"/
12 *evaluation studies as topic/
13 *reproducibility of results/
14 *"sensitivity and specificity"/
15 "quality control"/
16 (assess$ or authenticate$ or best practice$ or comparative or compare$ or comparing or comparison or compliance or complie$ or comply or concordan$ or confirm$ or correlate$ or correlation or discordan$ or evaluat$ or guideline$ or implement$ or parallel or performance or post validat$ or protocol$ or quality or reassess$ or reliability or reliably or reliable or repeat$ or reproducib$ or sensitivity or specificity or revalidat$ or re-validat$ or standard$ or validate$ or validity or validation or verification or verified or verify).ti,kf.
17 (assess$ or authenticate$ or best practice$ or comparative or compare$ or comparing or comparison or compliance or complie$ or comply or concordan$ or confirm$ or correlate$ or correlation or discordan$ or evaluat$ or guideline$ or implement$ or parallel or performance or post validat$ or protocol$ or quality or reassess$ or reliability or reliably or reliable or repeat$ or reproducib$ or sensitivity or specificity or revalidat$ or re-validat$ or standard$ or validate$ or validity or validation or verification or verified or verify).ab. /freq=2
18 or/9-16
19 or/9-17
20 fixatives/
21 formaldehyde/
22 edetic acid/
23 hydrochloric acid/
24 nitric acid/
25 formates/
26 acetates/
27 decalcification technique/
28 (air dried or AZF or borate or Bouin$ or cell block or Cellient or Christensen$ or citrate or cytology or cytologic or cytopathol$ or CytoLyt or CytoRich or decalcify or decalcified or decalcification or decalcifies or demineral$ or EDTA or ethanol or ethylenediaminetetraacetic or fixative$ or fixation or FFPE or formaldehyde or formalin$ or Hank$ or HCL or histogel or ischemic or liquid based or methylene glycol or methanol or multiplex staining or NBF or paraffin embedded or pre-analytic$ or preanalytic$ or Preservcyt or preservative$ or protease or reagent$ or RPMI or saline or stain$ method or stain$ protocol or stain$

intensity or positive stain$ or negative stain$ or SurePath or ThinPrep or touch print or Tripsin or TRIS or unfixed or antibody lot or antibody dilution or antigen retrieval or antigen detection or antibody clone or assay$ or immunostain$).ti,kf.
29 (air dried or AZF or borate or Bouin$ or cell block or Cellient or Christensen$ or citrate or cytology or cytologic or cytopathol$ or CytoLyt or CytoRich or decalcify or decalcified or decalcification or decalcifies or demineral$ or EDTA or ethanol or ethylenediaminetetraacetic or fixative$ or fixation or FFPE or formaldehyde or formalin$ or Hank$ or HCL or histogel or ischemic or liquid based or methylene glycol or methanol or multiplex staining or NBF or paraffin embedded or pre-analytic$ or preanalytic$ or Preservcyt or preservative$ or protease or reagent$ or RPMI or saline or stain$ method or stain$ protocol or positiv$ stain$ or negativ$ stain$ or stain$ intensity or SurePath or ThinPrep or touch print or Tripsin or TRIS or unfixed or antibody lot or antibody dilution or antigen retrieval or antigen detection or antibody clone or assay$ or immunostain$).ab. /freq=2
30 or/20-28
31 or/20-29
32 ("fit for use" or "fit for purpose").tw,kf.
33 ("expected purpose" or "expected use" or "specified purpose" or "specified use" or "intended purpose" or "intended use").tw,kf.
34 or/32-33
35 5 and 19
36 6 and 18
37 or/35-36
38 37 and 31
39 6 and 19 and 30
40 8 and 34
41 or/38-40
42 limit 41 to (english language and yr="2013 -Current")
43 animals/ not humans/
44 42 not 43
45 (animal* or rat or rats or dog or dogs or cat or cats or mice or mouse or murine or bovine or canine or porcine or monkey or pig or lungfish or hens or equine or buffalo or rodent or rodents or sows).ti.
46 (antibody or antibodies or human or humans or patient or patients).ti.
47 45 not 46
48 44 not 47
49 limit 48 to (case reports or comment or editorial or letter)
50 case report.ti.
51 49 or 50
52 limit 51 to (clinical study or comparative study or guideline or meta analysis or practice guideline or "systematic review" or technical report or validation studies)
53 51 not 52
54 48 not 53
55 remove duplicates from 54

EMBASE Search String #1

((((('immunohistochemistry'/mj OR 'immunoperoxidase staining'/mj OR immunohistochemistry:ti,kw OR immunohistochemical:ti,kw OR immunohistochemically:ti,kw OR immunoperoxidase:ti,kw OR immunocytochemical:ti,kw OR immunocytochistry:ti,kw) AND ('validation process'/de OR 'validation study'/de OR 'feasibility study'/de OR 'quality control'/mj OR 'evaluation study'/de OR 'reproducibility'/mj OR 'sensitivity or specificity'/mj OR 'quality control'/de OR 'good laboratory practice'/de OR 'instrument validation'/de OR 'total quality management'/de OR 'best practice':ti,ab,kw OR comparative*:ti,ab,kw OR

comparing:ti,ab,kw OR comparison:ti,ab,kw OR compliance:ti,ab,kw OR comply:ti,ab,kw OR complies:ti,ab,kw OR concordan*:ti,ab,kw OR confirm*:ti,ab,kw OR correlate*:ti,ab,kw OR discordan*:ti,ab,kw OR evaluat*:ti,ab,kw OR guideline*:ti,ab,kw OR implement*:ti,ab,kw OR 'parallel test*':ti,ab,kw OR performance:ti,ab,kw OR 'post validat*':ti,ab,kw OR protocol*:ti,ab,kw OR quality:ti,ab,kw OR reassess*:ti,ab,kw OR reliability:ti,ab,kw OR reliably:ti,ab,kw OR reliable:ti,ab,kw OR repeat*:ti,ab,kw OR reproducib*:ti,ab,kw OR revalidat*:ti,ab,kw OR 're validat*':ti,ab,kw OR standard*:ti,ab,kw OR validat*:ti,ab,kw OR validity:ti,ab,kw OR verification:ti,ab,kw OR verifie*:ti,ab,kw OR verify:ti,ab,kw)) OR (('immunohistochemistry'/mj OR 'immunoperoxidase staining'/de OR immunohistochemistry:ti,ab,kw OR immunohistochemical:ti,ab,kw) AND ('validation process'/de OR 'validation study'/de OR 'feasibility study'/de OR 'quality control'/mj OR 'evaluation study'/de OR 'reproducibility'/mj OR 'sensitivity or specificity'/mj OR 'quality control'/de OR 'good laboratory practice'/de OR 'instrument validation'/de OR 'total quality management'/de OR 'best practice':ti,kw OR comparative*:ti,kw OR comparing:ti,kw OR comparison:ti,kw OR compliance:ti,kw OR comply:ti,kw OR complies:ti,kw OR concordan*:ti,kw OR confirm*:ti,kw OR correlate*:ti,kw OR discordan*:ti,kw OR evaluat*:ti,kw OR guideline*:ti,kw OR implement*:ti,kw OR 'parallel test*':ti,kw OR performance:ti,kw OR 'post validat*':ti,kw OR protocol*:ti,kw OR quality:ti,kw OR reassess*:ti,kw OR reliability:ti,kw OR reliably:ti,kw OR reliable:ti,kw OR repeat*:ti,kw OR reproducib*:ti,kw OR revalidat*:ti,kw OR 're validat*':ti,kw OR standard*:ti,kw OR validat*:ti,kw OR validity:ti,kw OR verification:ti,kw OR verifie*:ti,kw OR verify:ti,kw))) AND (fixative/de OR formaldehyde/de OR 'decalcification'/exp OR 'air dried':ti,ab,kw OR fixative:ti,ab,kw OR fixation:ti,ab,kw OR azf:ti,ab,kw OR bouin*:ti,ab,kw OR 'cell block*':ti,ab,kw OR cellient:ti,ab,kw OR christensen*:ti,ab,kw OR cytolog*:ti,ab,kw OR cytopathol*:ti,ab,kw OR cytolyt:ti,ab,kw OR cytorich:ti,ab,kw OR decalcif*:ti,ab,kw OR demineral*:ti,ab,kw OR edta:ti,ab,kw OR ethanol:ti,ab,kw OR ffpe:ti,ab,kw OR formaldehyde:ti,ab,kw OR formalin*:ti,ab,kw OR hank*:ti,ab,kw OR histogel:ti,ab,kw OR ischemic:ti,ab,kw OR 'liquid based':ti,ab,kw OR 'methylene glycol':ti,ab,kw OR methanol:ti,ab,kw OR 'multiplex staining':ti,ab,kw OR ‘dual stain*’:ti,ab,kw OR ‘duo stain*’:ti,ab,kw OR nbf:ti,ab,kw OR 'paraffin embedded':ti,ab,kw OR picric:ti,ab,kw OR pre*analytic*:ti,ab,kw OR preservcyt:ti,ab,kw OR preservative*:ti,ab,kw OR protease:ti,ab,kw OR reagent*:ti,ab,kw OR rpmi:ti,ab,kw OR saline:ti,ab,kw OR 'stain* method*':ti,ab,kw OR 'stain* protocol*':ti,ab,kw OR ‘positive stain*’:ti,ab,kw OR ‘negative stain*’:ti,ab,kw OR ‘stain* intensity’:ti,ab,kw OR surepath:ti,ab,kw OR thinprep;ti,ab,kw OR 'touch print':ti,ab,kw OR tripsin:ti,ab,kw OR tris:ti,ab,kw OR unfixed:ti,ab,kw OR 'antibody lot':ti,ab,kw OR 'antibody dilution':ti,ab,kw OR 'antigen retrieval':ti,ab,kw OR 'antigen detection':ti,ab,kw OR 'antibody clone':ti,ab,kw OR assay*:ti,ab,kw OR immunostain*:ti,ab,kw OR laborator*:ti,ab,kw))

Embase Search String #2

(('fixative'/de OR 'formaldehyde'/de OR 'decalcification'/exp OR 'air dried':ti,kw OR fixative:ti,kw OR fixation:ti,kw OR azf:ti,kw OR bouin*:ti,kw OR 'cell block*':ti,kw OR cellient:ti,kw OR christensen*:ti,kw OR citrate:ti,kw OR cytolog*:ti,kw OR cytopathol*:ti,kw OR cytolyt:ti,kw OR cytorich:ti,kw OR decalcif*:ti,kw OR demineral*:ti,kw OR edta:ti,kw OR ethanol:ti,kw OR ffpe:ti,kw OR formaldehyde:ti,kw OR formalin*:ti,kw OR hank*:ti,kw OR histogel:ti,kw OR ischemic:ti,kw OR 'liquid based':ti,kw OR 'methylene glycol':ti,kw OR methanol:ti,kw OR 'multiplex staining':ti,kw OR ‘dual stain*’:ti,kw OR ‘duo stain*’:ti,kw OR nbf:ti,kw OR 'paraffin embedded':ti,kw OR picric:ti,kw OR pre*analytic*:ti,kw OR preservcyt:ti,kw OR preservative*:ti,kw OR protease:ti,kw OR reagent*:ti,kw OR rpmi:ti,kw OR saline:ti,kw OR 'stain* method*':ti,kw OR 'stain* protocol*':ti,kw OR 'positive stain*':ti,kw OR 'negative stain*':ti,kw OR 'stain* intensity':ti,kw OR surepath:ti,kw OR thinprep;ti,kw OR 'touch print':ti,kw OR tripsin:ti,kw OR tris:ti,kw OR unfixed:ti,kw OR 'antibody lot':ti,kw OR 'antibody dilution':ti,kw OR 'antigen retrieval':ti,kw OR 'antigen detection':ti,kw OR 'antibody clone':ti,kw OR assay*:ti,kw OR immunostain*:ti,kw OR laborator*:ti,kw) AND ('immunohistochemistry'/mj OR 'immunoperoxidase staining'/de OR immunohistochemi:ti,ab,kw OR immunocytochemi*:ti,ab,kw) AND ('validation process'/de OR 'validation study'/de OR 'feasibility study'/de

OR 'quality control'/mj OR 'evaluation study'/de OR 'reproducibility'/mj OR 'sensitivity or specificity'/mj OR 'quality control'/de OR 'good laboratory practice'/de OR 'instrument validation'/de OR 'total quality management'/de OR 'best practice':ti,ab,kw OR comparative*:ti,ab,kw OR comparing:ti,ab,kw OR comparison:ti,ab,kw OR compliance:ti,ab,kw OR comply:ti,ab,kw OR complie*:ti,ab,kw OR concordan*:ti,ab,kw OR confirm*:ti,ab,kw OR correlate*:ti,ab,kw OR discordan*:ti,ab,kw OR evaluat*:ti,ab,kw OR guideline*:ti,ab,kw OR implement*:ti,ab,kw OR 'parallel test*':ti,ab,kw OR performance:ti,ab,kw OR 'post validat*':ti,ab,kw OR protocol*:ti,ab,kw OR quality:ti,ab,kw OR reassess*:ti,ab,kw OR reliability:ti,ab,kw OR reliably:ti,ab,kw OR reliable:ti,ab,kw OR repeat*:ti,ab,kw OR reproducib*:ti,ab,kw OR revalidat*:ti,ab,kw OR 're validat*':ti,ab,kw OR standard*:ti,ab,kw OR validat*:ti,ab,kw OR validity:ti,ab,kw OR verification:ti,ab,kw OR verifie*:ti,ab,kw OR verify:ti,ab,kw))

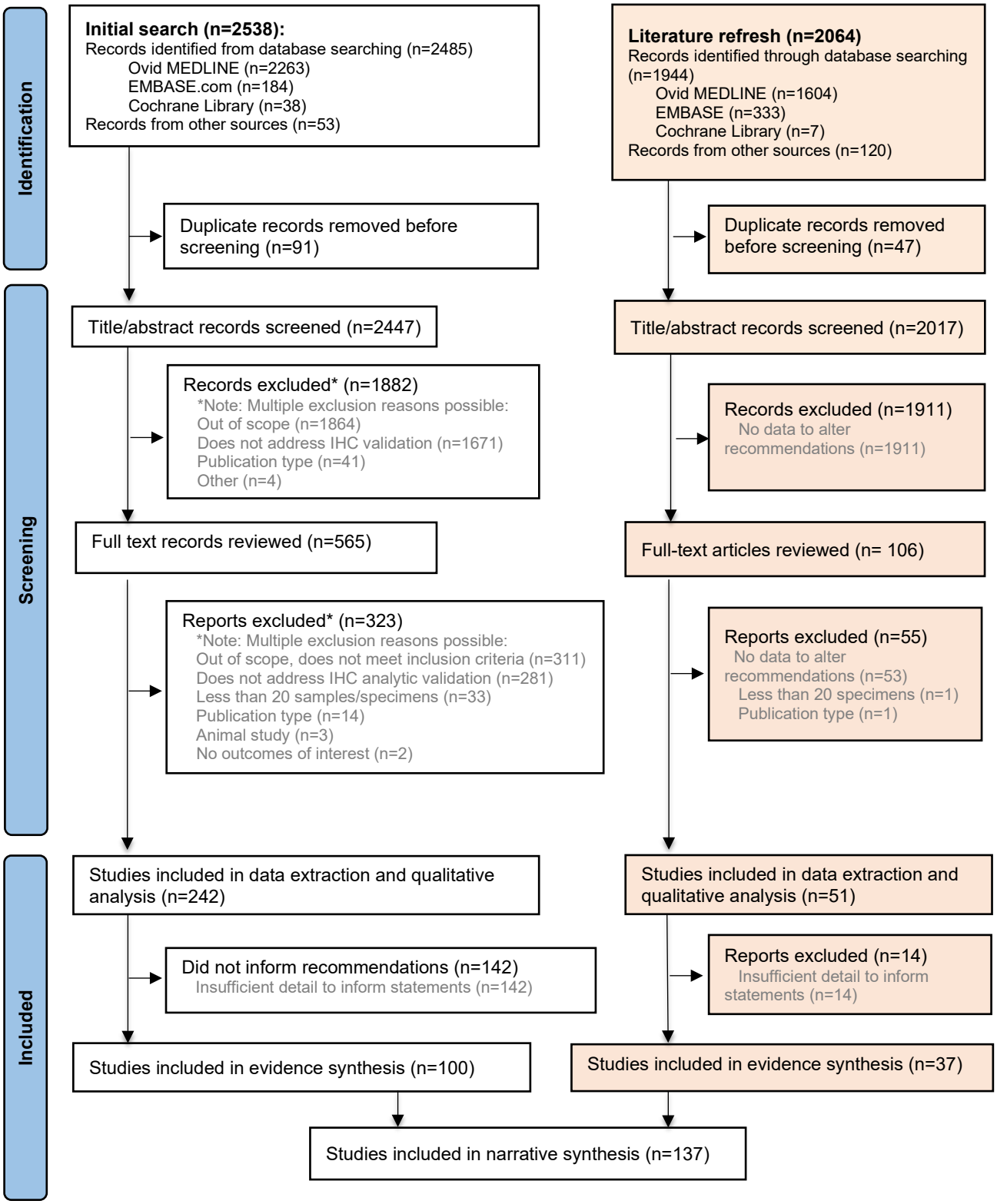
Embase Search String #3:

('immunohistochemistry'/de OR 'immunoperoxidase staining'/de OR immunohistochemi*:ti,ab,kw OR immunocytochemi*:ti,ab,kw OR immunoperoxidase:ti,ab,kw) AND ('fit for use':ti,ab,kw OR 'fit for purpose':ti,ab,kw OR 'expected use':ti,ab,kw OR 'expected purpose':ti,ab,kw OR 'specified use':ti,ab,kw OR 'specified purpose':ti,ab,kw OR 'intended use':ti,ab,kw OR 'intended purpose':ti,ab,kw)

Cochrane Search String:

((immunohistochemistry OR immunohistochemical)):ti,ab,kw AND ((validate or validating or validation)):ti,ab,kw

Supplemental Figure 2: Literature Review Flow Diagram



Adapted from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

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