Immunohistochemical (IHC) Marker Template
For Integral Markers in Clinical Trials

This is a template to describe the analytical and clinical performance of an assay that is essential for performance of a trial. It will be used to assess whether assays are ready for use in a trial by Disease Steering Committees and CTEP. The FDA may also use it to evaluate integral assays and diagnostics for their pre-IDE evaluation. Not all parameters may be known a priori. Please enter as much information as you can and N/A for not available or applicable where appropriate.

This template requires detailed information that may be known only by laboratorians, scientists who work in clinical laboratories, and should be collaborating closely with clinical trialists. Please be sure to collect the appropriate responses before filling out this form. The template has the following sections with information needed from trialists and laboratorians:

1. Assay, Patient and Specimen Information –Trialists and Laboratorians

2. Primary Antibody Characteristics – Laboratorians

3. Design of Immunohistochemical Assay - Laboratorians

4. Assay Performance – Laboratorians

5. Laboratory Information – Trialists and Laboratorians

Section 1. Assay, Patient and Specimen Information

A. Name of marker (Please use HUGO gene or protein name for molecular marker or the Atlas for Genetics in Hematology and Oncology for cytogenetic or FISH markers)

HUGO Site: http://www.genenames.org/ -
Atlas Site: http://atlasgeneticsoncology.org/index.html

B. How will assay and its marker be used in clinical trial?

○ **Integral Marker** ○ **Integrated Marker** ○ **Research Marker**

- **Integral markers are required for the trial to proceed (e.g., patient eligibility, assignment to treatment, stratification, risk classifier or medical decision-making - often requires performance in a CLIA laboratory).**
- **Integrated markers are performed on all or a statistical subset of patients but are not used for medical decision-making.**
- **Research markers are all other assays and commonly referred to as correlative research.**
- **For other definitions, please see References at end of form.**

B1. Assay Purpose

Treatment Assignment

C. Assay type

IHC

D. Will assay be performed in a Central Reference CLIA lab, multiple CLIA-certified labs, or research labs?

○ **Central Reference CLIA Lab** ○ **Multiple CLIA Labs** ○ **Research Labs**

E. Anatomic source of specimens (organ site)

E1. Type of Specimen

Skin

All Comers
Anus
Appendix
Bile Duct

E2. Tissue collection

Not Specified

F. Patient conditions or co-morbidities that may affect assay and must be noted:

G. Preanalytic Specimen Requirements

G1. Maximum Warm ischemia time (=time from cutting blood supply to removal from body) allowed in minutes if known:

Unknown

G2. Maximum Cold ischemia time (=time until specimen fixed/frozen after removal from body) allowed in minutes if known:

Unknown

G3. Type of stabilization of Specimen: ◯ **fixed** ◯ **frozen** ◯ **both**

G3a If fixed, what fixation buffer to be used? Other
Bouin's
10% Neutral Buffered Formalin

G3b. If Other fixative, what is it? (free text)

G3c What is shortest fixation time allowed (Hours or fraction thereof)

G3d What is longest fixation time allowed (Hours or fraction thereof)

G3e If frozen, how will specimen be frozen:

Cryopreserved with controlled rate freezing

H. How will specimens be stored?

Room Temperature

I. Specimen size to be stored 1444 **length width height in cm**

J. Tissue section thickness on slide in microns

K. Antigen retrieval solution/procedures

Section 2. Primary Antibody Characteristics

A. Source of primary antibody (purchased from xxx as lot # xxx, or generated in house, etc.)

B. What was the immunogen (e.g., peptide, oligosaccharide, phosphorylated protein, other)?

◯ **Protein** ◯ **Peptide** ◯ **Oligosaccharide** ◯ **Phosphorylated Protein** ◯ **Other**

B1. Please describe if Other

C. Species of immunogen (e.g., human or mouse gene product)

Human

D. Are there specific isoform(s) of the immunogen that are recognized (e.g., one or all isoforms or unknown)?

◯ **One Isoform** ◯ **All isoforms** ◯ **Unknown**

E. Preparation of immunogen (e.g., purified protein, recombinant, synthetic peptide or oligosaccharide)

◯ **purified protein** ◯ **recombinant** ◯ **synthetic peptide** ◯ **oligosaccharide**

F. Other attributes of primary antibody (e.g., mono- or polyclonal)

◯ **Monoclonal** ◯ **Polyclonal**

F1. What species: Mouse

F1a. If other species, what is it? Include chicken

G. How was the antibody specificity demonstrated?

Western Blot

G1. Please specify if Other

H2. Are there band(s) at the expected mass(es) on Western blot?

◯ Yes ◯ No ◯ Unknown

H2a. If not, please explain

H3. Is immunostaining abolished in knock out/knock-down cells or with epitope-absorbed antibody?

◯ Yes ◯ No ◯ Unknown

H4. Is immunostaining abolished when antibody absorbed or blocked with epitope?

◯ Yes ◯ No ◯ Unknown

I. What is the targeted organ/tissue/cell (e.g., normal melanocytes? breast ductal carcinoma)?

I1. What non-targeted organ/tissue/cell is also stained?

J. Have any cross-reactive proteins or peptides been identified that may confound interpretation of IHC?

◯ Yes ◯ No ◯ Unknown

J1. If yes and known, what are they?

K. Is antigen stable when the period between tissue sectioning and staining is

◯ <7 days ◯ 7-30 days ◯ >30 days ◯ Not Known

Section 3. Design of Immunohistochemical Assay

A. Assay Design (Complete assay details are needed if multiple labs will perform the assay).

A1. Describe the platform of the assay, e.g. instrument (manufacturer, model, UDI number if known)

A1a. Platform

A1b. Manufacturer

A1c. Model Number

A1d. UDI Number (Universal Device Number)

A1e. Is the platform cleared or approved by the FDA

◯ Yes ◯ No ◯ Unknown

A2. Is there an SOP?

◯ Yes ◯ No ◯ Unknown

A2a. Is the SOP attached as an Appendix?

◯ Yes ◯ No ◯ Unknown

B. Type of Immunoassay

B1. Is the assay qualitative, semiquantitative or quantitative

◯ Qualitative ◯ Semiquantitative ◯ Quantitative

B1a. If an image analyzer is used, what manufacturer and model was used?

B1b. Is it cleared or approved by the FDA

◯ Yes ◯ No ◯ Unknown

B2. Nature of reporter signal

B3. Assay method (e.g. direct, indirect, 3-step immunoperoxidase assay)

◯ Direct ◯ Indirect ◯ 3-step Immunoperoxidase ◯ Other

If other, please specify

B3a. What secondary reagent(s) is used for the indirect or 3-step assay

C. Are there positive and negative controls for the assay

◯ Yes ◯ No ◯ Unknown

C1. If there are controls, what are they?

D. Specimen size – What is the smallest specimen that can be analyzed by the assay in cm?

cm

D1. Is the minimum specimen size determined by a particular characteristic of the tissue?

◯ **Yes** ◯ **No** ◯ **Unknown**

D1a. If so, is it ◯ **Number of cell nuclei** ◯ **Nuclear area** ◯ **Cytoplasmic area** ◯ **Other**

D1b. Please specify if Other

Section 4. Assay Performance

A. Details regarding how the analyte is measured

A1. What statistical test(s) were used to validate the assay results.

A2. How was a clinically relevant threshold selected?

Literature

A3. Were results obtained on retrospective or prospective data sets?

Retrospective **Sample Size**

Prospective

A3a. Training sets or other validation method Separate Training & Validation Sets

A4. What is the cut-off?

A5. How well was the cut-off validated before using it in these trials?

A6. Were assay conditions standardized to minimize variance, e.g., automated tissue processors and/or stainers)?

◯ **Yes** ◯ **No** ◯ **Unknown**

A6a. If yes, what tissue processor/stainer was used?

A7. If calibrators or controls were used, were they stained separately with each batch of slides, included on each slide or internal controls?

A7a. Were calibrators/controls used?

◯ **Yes** ◯ **No** ◯ **Unknown**

A7b. Were the controls stained as separate slides with slides?

◯ **Yes** ◯ **No** ◯ **Unknown**

OR A7c. Were the controls included in each slide and stained as internal controls?

◯ **Yes** ◯ **No** ◯ **Unknown**

OR A7d. Were the controls not stained in each staining run?

◯ **Yes** ◯ **No** ◯ **Unknown**

B. Reproducibility of assay

B1. Was reproducibility assessed?

◯ **Yes** ◯ **No** ◯ **Unknown**

B1a. If yes, please describe the specimen type(s) used

B1b. If not, please explain

B2. How many replicates were done?

B3. What is the intra-lab reproducibility (%CV)?

B4. What is the inter-lab reproducibility (same specimens, different lab, number of different technicians)?

B4a. How many on the same specimens?

B4b. How many different labs?

B4c. How many different technicians?

B4d. What types of specimens (e.g., tissue sections, TMA)?

B4e. Over how many different days?

B4f. How many readers?

B5. What is the agreement between readers?

B5a. How are differences resolved?

Other (please specify)

C. Image Measurement

C1. What strategy was used to select the fields to be analyzed?

C2. How was a threshold to distinguish positive from negative determined?

C3. How were the cells of interest distinguished from other cells?

C4. Was reference material used to generate a standard curve?

◯ **Yes** ◯ **No** ◯ **Unknown**

C4a. What was the reference material?

C4b. Has it been cleared by the FDA?

◯ **Yes** ◯ **No** ◯ **Unknown**

D. Assay Discrimination

D1. What is the accuracy of the assay for detecting the analyte?

D2. How are staining and tissue artifacts identified and handled (especially if image analysis is used)?

Section 5. Laboratory Information

A. Is the lab a research or clinical lab?

◯ **Research** ◯ **Clinical**

B. Does the lab meet GLP standards

◯ **Yes** ◯ **No** ◯ **Unknown**

C. What is the training and experience of the Technicians/Operators?

References

Section	Ref #	Citation
1.	20215558	Dancey JE, Dobbin KK, Groshen S, Jessup JM, Hruszkewycz AH, Koehler M, Parchment R, Ratain MJ, Shankar LK, Stadler WM, True LD, Gravell A, Grever MR; Biomarkers Task Force of the NCI Investigational Drug Steering Committee. Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin Cancer Res. 2010 Mar 15;16(6):1745-55.
1.A	18327244	Deutsch EW, Ball CA, Berman JJ, Bova GS, Brazma A, Bumgarner RE, Campbell D, Causton HC, Christiansen JH, Daian F, Dauga D, Davidson DR, Gimenez G, Goo YA, Grimmond S, Henrich T, Herrmann BG, Johnson MH, Korb M, Mills JC, Oudes AJ, Parkinson HE, Pascal LE, Pollet N, Quackenbush J, Ramialison M, Ringwald M, Salgado D, Sansone SA, Sherlock G, Stoeckert CJ Jr, Swedlow J, Taylor RC, Walashek L, Warford A, Wilkinson DG, Zhou Y, Zon LI, Liu AY, True LD. Minimum information specification for in situ hybridization and immunohistochemistry experiments (MISFISHIE). Nat Biotechnol. 2008 Mar;26(3):305-12.
1.B OBBR	https://brd.nci.nih.gov/BRN/brnHome.seam?conversationId=12427	
1.F1	12414521	Dash A, Maine IP, Varambally S, Shen R, Chinnaiyan AM, Rubin MA. Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. Am J Pathol. 2002 Nov;161(5):1743-8.
1.F2	19734848	Khoury T, Sait S, Hwang H, Chandrasekhar R, Wilding G, Tan D, Kulkarni S. Delay to formalin fixation effect on breast biomarkers. Mod Pathol. 2009 Nov;22(11):1457-67.
1.F1-2.	19415952	Middleton LP, Price KM, Puig P, Heydon LJ, Tarco E, Sneige N, Barr K, Deavers MT. Implementation of American Society of Clinical Oncology/College of American Pathologists HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence in situ hybridization concordance and decreases the number of inconclusive cases. Arch Pathol Lab Med. 2009 May;133(5):775-80.
2.E	20093391	Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. J Mol Diagn. 2010 Mar;12(2):169-76.
	20359301	Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D. Antibody validation. Biotechniques. 2010 Mar;48(3):197-209.
	18796405	Pradidarcheep W, Labruyère WT, Dabhoiwala NF, Lamers WH. Lack of specificity of commercially available antisera: better specifications needed. J Histochem Cytochem. 2008 Dec;56(12):1099-111.

2.E7 NCBI Blast Search http://blast.ncbi.nlm.nih.gov/Blast.cgi

4. 20524870 Fitzgibbons PL, Murphy DA, Hammond ME, Allred DC, Valenstein PN. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. Arch Pathol Lab Med. 2010 Jun;134(6):930-5.

ISBN978-0-387-72805-6 Discrete Multivariate Analysis: Theory and Practice. Authors: Paul W. Holland (Author)Stephen E. Fienberg (Author)Yvonne M. Bishop (Author)F. Mosteller (Contributor)R.J. Light (Contributor). Springer. 30 July 2007. English. Paperback, 558 pages

18829475 Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. Clin Cancer Res. 2008 Oct 1;14(19):5967-76.

Appendix to CLSI document IL-28a

Appendix to CLSI document IL-28a

4.B5 2189948 Cicchetti DV, Feinstein AR. High agreement but low kappa: II. Resolving the paradoxes. J Clin Epidemiol. 1990;43(6):551-8.

4.B 11459866 Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001 Jul 18;93(14):1054-61.

4.B 20586616 Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010 Jul;134(7):e48-72.

4.8 2578140 Gross DS, Rothfeld JM. Quantitative immunocytochemistry of hypothalamic and pituitary hormones: validation of an automated, computerized image analysis system. J Histochem Cytochem. 1985 Jan;33(1):11-20.