LOI/Concept/Protocol #: Protocol Investigator:

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

C. Assay type

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

E2. Tissue collection

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:

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

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

G3a If fixed, what fixation buffer to be used?

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:

H. How will specimens be stored?

I. Specimen size to be stored 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)

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:

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

G. How was the antibody specificity demonstrated?

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?

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

Sample Size

A3a. Training sets or other validation method

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?

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
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Appendix to CLSI document IL-28a

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