Immunohistochemical (IHC) Marker Template

For Integrated 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 Nomenclature Committee (HGNC)* gene or protein name for molecular marker or the *Atlas of Genetics and Cytogenetics in Oncology and Haematology* for cytogenetic or FISH markers)

HGNC Site: <http://www.genenames.org/>

Atlas Site: <http://atlasgeneticsoncology.org/index.html>

 Marker name \_\_\_\_\_\_\_\_\_

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 \_\_ Eligibility Criterion

\_\_ Stratification Factor \_\_ Other (Specify) \_\_\_\_\_\_\_\_

C. Assay type

 \_\_ IHC \_\_ ISH \_\_ FISH \_\_ ELISA \_\_ Microarray \_\_ RT-PCR

 \_\_ Other (Specify) \_\_\_\_\_\_\_

D. Will the assay be performed in a central reference CLIA lab, multiple CLIA-certified labs, or in

 research labs?

 \_\_ Central Reference CLIA Lab \_\_ Multiple CLIA Labs \_\_ Research Labs

E. Anatomic source of specimens (organ site) \_\_\_\_\_\_\_\_

E1. Type of Specimen \_\_ N/A

\_\_ ascites \_\_ bone marrow \_\_ cell \_\_ normal \_\_ plasma \_\_ serum

\_\_ blood \_\_ buccal mucosa \_\_ CSF \_\_ tumor \_\_ skin \_\_ pleural fluid

\_\_ urine \_\_ Other (Specify) \_\_\_\_\_\_\_\_

E2. Tissue collection

 \_\_ mandatory (must be performed on trial)

 \_\_ mandatory on consent (must be performed when consent obtained)

 \_\_ voluntary

 \_\_ 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 \_\_ Known \_\_ Unknown

*Please specify if known* \_\_\_\_ Minutes

G2. Maximum Cold ischemia time (= time from removal from body to being frozen or put into preservative) allowed in minutes if known \_\_ Known \_\_ Unknown

 *Please specify if known* \_\_\_\_\_ Minutes

G3. Type of stabilization of Specimen: \_\_ Fixed \_\_ Frozen \_\_ Both

G3a. If fixed, what fixation buffer? \_\_ Bouin’s \_\_ 10% Neutral Buffered formalin

 \_\_ Other (Specify) \_\_\_\_\_\_\_\_

G3a1. What is the shortest fixation time allowed (Hours or fractions thereof)?

 \_\_\_\_\_\_\_\_

G3a2. What is the longest fixation time allowed (Hours or fractions thereof)?

 \_\_\_\_\_\_\_\_

G3b. If frozen, how will the specimen be frozen?

 \_\_ Flash Frozen (to -80°C)

 \_\_ Embedded in OCT and then frozen

 \_\_ Cryopreserved with controlled rate freezing

 H. How will the specimens be stored?

 \_\_ -20°C

 \_\_ -80°C

 \_\_ -100°C to -130°C

 \_\_ Vapor Phase Liquid Nitrogen

 \_\_ 4°C

 \_\_ Room Temperature

I. Specimen size to be stored \_\_Inches \_\_ Centimeters

 Length \_\_\_\_\_\_\_

 Width \_\_\_\_\_\_\_

 Height \_\_\_\_\_\_\_

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

 \_\_ Protein \_\_ Peptide \_\_ Oligosaccharide \_\_ Phosphorylated Protein

 \_\_ Other (describe) \_\_\_\_\_\_

B1. Species of immunogen (e.g., human or mouse gene product)

 \_\_ Human \_\_ Mouse \_\_ Recombinant \_\_ Other (Specify) \_\_\_\_\_\_\_\_

B2. Are there specific isoform(s) of the immunogen that are recognized (e.g., one or all isoforms or unknown)?

 \_\_ One Isoform \_\_ All Isoforms \_\_ Unknown

B3. Preparation of immunogen

 \_\_ Purified Protein \_\_ Recombinant \_\_ Synthetic Peptide \_\_ Oligosaccharide

C. Other attributes of the primary antibody

C1. \_\_ Monoclonal \_\_ Polyclonal

C2. \_\_ Human

 \_\_ Mouse

 \_\_ Rabbit

 \_\_ Goat

 \_\_ Horse

 \_\_ Chicken

 \_\_ Other (specify) \_\_\_\_\_\_\_\_

D. How was the antibody specificity demonstrated?

\_\_ IHC \_\_ Western Blot \_\_ Immunoprecipitation \_\_ Immunocompetition

\_\_ Other (specify) \_\_\_\_\_\_\_\_

D1. Are there band(s) at the expected mass(es) on a Western blot?

 \_\_ Yes \_\_ No \_\_Unknown

 If no, please explain \_\_\_\_\_\_\_\_

D2. Is immunostaining abolished in knock out/knock-down cells or with epitope-absorbed antibody?

 \_\_ Yes \_\_ No \_\_ Unknown

D3. Is immunostaining abolished when antibody absorbed or blocked with epitope?

 \_\_ Yes \_\_ No \_\_ Unknown

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

 \_\_\_\_\_\_\_\_

E1. What non-targeted organ/tissue/cell is also stained?

 \_\_\_\_\_\_\_\_

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

 \_\_ Yes \_\_ No \_\_ Unknown

If yes and known, what are they? \_\_\_\_\_\_\_\_\_

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

 \_\_ <7 days \_\_ 7-30 days \_\_ >30 \_\_ 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, semi-quantitative or quantitative?

 \_\_ Qualitative \_\_ Semi-quantitative \_\_ 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 the reporter signal

 \_\_\_\_\_\_\_\_

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

 \_\_ direct \_\_ indirect \_\_ 3-step Immunoperoxidase

 \_\_ other (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. What is the smallest specimen that can be analyzed by the assay? \_\_ 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 (specify) \_\_\_\_\_

# Section 4. Assay Performance

A. Details regarding how the analysis is measured

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

 \_\_\_\_\_\_\_\_

A2. How was a clinically relevant threshold selected?

 \_\_ Literature \_\_ Pilot Clinical Study \_\_ Medical Practice Guidelines

 \_\_ Non-clinical data (e.g., *in vitro* or *in vivo* animal) \_\_ Other (specify) \_\_\_\_\_\_\_

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

 \_\_ Retrospective \_\_ Prospective Sample Size \_\_\_\_\_\_\_

 A3a. Training sets or other validation method

 \_\_ Separate Training & Validation Sets \_\_ Other Method (specify) \_\_\_\_\_

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 strainers)?

 \_\_ Yes \_\_ No \_\_ Unknown

 If yes, what tissue processor/stainer was used? \_\_\_\_\_

A7. Were calibrators/controls used? \_\_ Yes \_\_ No \_\_ Unknown

A7a. Were the controls stained as separate slides with slides?

 \_\_ Yes \_\_ No \_\_ Unknown

A7b. Were the controls included in each slide and stained as internal controls?

 \_\_ Yes \_\_ No \_\_ Unknown

A7c. Were the controls not stained in each staining run?

 \_\_ Yes \_\_ No \_\_ Unknown

B. Reproducibility of assay

B1. Was reproducibility assessed?

 \_\_ Yes \_\_ No \_\_ Unknown

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

 \_\_\_\_\_\_\_\_

If no, 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, and 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?

 \_\_ Different runs of the same assay

 \_\_ Different runs of another assay of the same technology

 \_\_ Different runs of another assay of a different technology

 \_\_ Different reading by the same reader or instrument

 \_\_ Different reading by a different reader or instrument

 \_\_ Panel or arbitration

 \_\_ 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. Will the assay be performed in 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 Technician/Operators? \_\_\_\_\_\_\_\_

# References

(Section, Ref #, Citation)

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Appendix to CLSI document IL-28a

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