**DNA-based Mutation Assays**

This is a template for use in outlining the known status of an assay that is to be used in a trial. The Clinical Laboratory Scientists who will be performing the tests should fill out this form. Not all parameters will be known a priori. Please enter as much information as you can. Enter N/A for not available or applicable where appropriate. This template is intended only for DNA-based somatic mutations and not for RNA-based mutations. It is also primarily intended for assays for markers that are integral for a trial. The information provided will be useful for an FDA pre-IDE review.

It is recommended that Jennings et. al. Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests. Arch Pathol Lab Med 133:743-755, 2009 be read as a reference before completing this template.

| **Section** | **Heading** |
| --- | --- |
| **1** | **Assay, Patient and Specimen Parameters** |
| **2** | **Design of Mutation Assay** |
| **3** | **Assay Performance** |
| **4** | **Laboratory that is performing this assay** |

# Assay, Patient and Specimen Parameters

A. Name of the Mutated gene(s) Gene ID that are measured, please follow nomenclature for mutation as supported by the Association of Molecular Pathology and the NCI EVS – example below:

Gene 1 \_\_\_\_\_\_\_\_

Gene 2 \_\_\_\_\_\_\_\_

Gene 3 \_\_\_\_\_\_\_\_

Gene 4 \_\_\_\_\_\_\_\_

Gene 5 \_\_\_\_\_\_\_\_

Gene 6 \_\_\_\_\_\_\_\_

Gene 7 \_\_\_\_\_\_\_\_

Please Use HUGO nomenclature for Gene ID (<http://www.genenames.org>)

Then please use the following format to identify the specific mutation:

Gene ID RefSeq#.Version c. nt # reference nt>altered nt (p. ref AA>mut AA) Example: BRAF NM\_004333.4 c. 1799 T>A (p.Val600Glu) N.B.: version number for refseq reference is critical because allows traceability

If performing a panel of more than 7 mutations, please provide in spreadsheet as an appendix.

B. How will assay and its marker be used in the clinical trial (Integral, Integrated, or Research)

 \_\_ Integral \_\_ Integrated \_\_ Research

C. Assay Purpose in Study

 \_\_ Treatment Assignment

 \_\_ Eligibility Criterion

 \_\_ Stratification

 \_\_ Retrospective Research

 \_\_ Prospective Research

D. Tissue Collection Consent Method under which samples are (were) obtained

 \_\_ Mandatory \_\_ Voluntary

E. Pre-Analytic Variables

E1. Ischemic Time From Collection to Specimen Processing or Fixation

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

Maximum Cold ischemia time (= time from removal from body to freezing or putting into preservative) allowed in minutes if known: \_\_\_\_\_\_\_\_\_

(If not known enter 99; If considered not important for assay, enter 98 for answers to previous 2 questions)

 E2. Type of Specimen

 \_\_ Feces

 \_\_ Skin

 \_\_ Ascites

 \_\_ Bone Marrow

 \_\_ CSF

 \_\_ Serum

 \_\_ Tumor

 \_\_ Buccal Mucosa

 \_\_ Urine

 \_\_ Pleural Fluid

 \_\_ Normal Tissue

 \_\_ Cell

 \_\_ Blood

 \_\_ Plasma

 \_\_ Other

 \_\_ None

F3. If sample is fluid (blood, ascites, pleural, cyst or other fluid), how was sample initially stabilized?

 \_\_ EDTA

 \_\_ Heparin

 \_\_ RNA preservative

 \_\_ Acid Citrate Dextrose (ACD)

 \_\_ Other

 \_\_ Not Applicable

F4. Type of stabilization of specimen

 \_\_ Fixed

 \_\_ Frozen

 \_\_ Both

 F4a. If fixed, was 10% Neutral Buffered Formalin Used?

 \_\_ Yes

 \_\_ No

 \_\_ Unknown

 F4b. If other fixatives are acceptable, what are they? \_\_\_\_\_\_\_\_

F4c. How long was the sample fixed?

 \_\_ <48 Hours

 \_\_ 48-96 Hours

 \_\_ >96 Hours

 \_\_ Not Known

F4d. If frozen, how was specimen frozen?

 \_\_ Flash Frozen

 \_\_ Embedded in OCT and then frozen

 \_\_ Controlled rate cryopreservation

 \_\_ Not Known

F4e. If frozen, at what temperature do you store the specimen?

 \_\_ -20 Degrees Celsius

 \_\_ -80 Degrees Celsius

 \_\_ -100 to -130 Degrees Celsius

 \_\_ Vapor Phase Liquid Nitrogen

 \_\_ 4 Degrees Celsius

 \_\_ Room Temperature

G. Specimen Characteristics

 G1. What type of specimen do you analyze (check all that apply)?

 \_\_ Section on slide

 \_\_ Section rolls

 \_\_ Punch Biopsy

 \_\_ Frozen Tissue

 \_\_ Cells

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

G2. Do you record the size/mass of specimen or number of cells that you use for analysis?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 G2a. Please indicate which characteristic is most important for specimen:

 \_\_ Size in cm

 \_\_ Mass in mg

 \_\_ Cell Number

G2b. Please give the minimum value (as appropriate): Size (Diameter in cm), Mass (mg), or Cells (Number) \_\_\_\_\_\_\_\_

G3. Is an adjacent tissue section stained and examined by H&E?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

G3a. If yes, was it used to assess cellularity and tumor content?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

G3b. Do you keep reference images of the H&E section?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 G4. Tumor content of specimen

 G4a. How is tumor content reported?

 \_\_ % Cells that are tumor cells

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

G4b. How is tumor content determined?

 \_\_ Flow cytometry

 \_\_ Digital imaging software

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

 G4c. What is the minimum acceptable % tumor content/cellularity?

 \_\_ <1%

 \_\_ 1 – 10%

 \_\_ 10 – 20%

 \_\_ 20 – 40%

 \_\_ >40%

G5. Do you enrich the sample for tumor cells?

 \_\_ Yes

 \_\_ No

 \_\_ Occasionally

 \_\_ Not Known

G5a. If so, what method did you use?

 \_\_ macrodissection (on a slide)

 \_\_ core punch sample

 \_\_ flow sorting

 \_\_ laser microdissection

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

**2. Design of Mutation Assay**

A. Describe the assay platform (please attach the complete SOP as an Appendix if you have one (including reagent details (lab manufactured protocols and commercial product/kit numbers & vendor))

 \_\_ Sanger sequencing

 \_\_ Pyrosequencing

 \_\_ PCR

 \_\_ Mass Spec

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

A1. Does the assay use molecular methods to enhance detection of mutations in a heterogeneous specimen?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

A1a. If so, what are the methods?

 \_\_ PNA-clamping

 \_\_ minimal tumor content

 \_\_ Digital PCR

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

A2. Is assay instrument (sequencer or PCR machine) tested for calibration or performance?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

A2a. If so, please provide the protocols as an Appendix if not included in the SOP \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 A2b. If not, how is confidence obtained that assay performs as intended?

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

B. DNA quality

B1. Does your lab use an automated or semi-automated method to extract DNA?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

B1a. If yes, which instrument do you use?

 \_\_ Qiagen BioRobot MDx Workstation

 \_\_ Qiagen EZ1

 \_\_ Roche MagNa Pure 96 System

 \_\_ Roche MagNa Pure LC 2.0 System

 \_\_ Roche MagNa Pure LC Compact System

 \_\_ Promega Maxwell 16

 \_\_ Autogen Gene Prep

 \_\_ QIAxtractor CAS 1820

 \_\_ Other

B1b. If your lab uses a manual method, does it use a commercially available kit?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

B1c. If yes, which reagents do you use?

 \_\_ Qiagen DNeasy Blood & Tissue Kits

 \_\_ Invitrogen ChargeSwitch gDNA Mini and Micro Tissue Kits

 \_\_ GenomicPrep Cells and Tissue DNA Isolation Kit

 \_\_ Roche DNA Isolation Kit for Cells and Tissues

 \_\_ Gentra puregene Tissue kit

 \_\_ Other

 B1ci. If another kit or reagents, what is (are) they? \_\_\_\_\_\_\_\_

B1d. If not, please check all reagents included in your DNA extraction:

 \_\_ Proteinase K

 \_\_ Boiling

 \_\_ Chelex

 \_\_ Phenol/chloroform

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

C. Do you assess DNA Concentration?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 C1. If you do, how is DNA concentration assessed?

 \_\_ Spectrophotometry

 \_\_ Fluorometry

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

D. Assay Quality Assessment

D1. Do you assess DNA purity?

 \_\_ Yes

 \_\_ No

 D2. How is DNA purity assessed?

 \_\_ Spectrophotometry (260/280 and/or 260/230)

 \_\_ Fluorometry

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

D3. Do you assess DNA integrity? \_\_\_\_\_

D3a. How is DNA integrity assessed?

 \_\_ Amplification of a control

 \_\_ Agilent Bioanalyzer

 \_\_ BioRad Experion

 \_\_ Gel Electrophoresis

 \_\_ PCR product

 \_\_ Other

D4. If second/next generation sequencing is used, is there a library preparation step?

\_\_ Yes

\_\_ No

\_\_ Unknown

\_\_ Not Second/Next Generation Sequencing

E. Data analysis

E1. How are raw data normalized, filtered, analyzed and reported? \_\_\_\_\_\_\_\_

 E1a. Provide detailed SOPs for data analysis \_\_\_\_\_\_\_\_

E1b. What software and version is used? \_\_\_\_\_\_\_\_

E1c. Are these limits set for reportable signal (e.g. maximum allowable Ct, parameters for somatic mutation calling by sequencing)

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 E2. If limits of reportable signal have been set, describe how these were determined.

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

E3. How is assay result reported (e.g. positive for mutation or negative for mutation)? OR, “report as low or high level”

 \_\_ Positive for mutation

 \_\_ Negative for mutation

 \_\_ Report as high or low value

 E4. Are there instructions for reasons why a sample should be repeated?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

E4a. If so, what are the instructions? \_\_\_\_\_\_\_\_

**3. Assay Performance**

A. Accuracy (Closeness to “true”)

 A1. Was accuracy established using reference material?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

A2. If yes, what reference material was used? \_\_\_\_\_\_\_\_

A3. Was accuracy established by comparison to a reference method(s)?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

A4. If yes, which method(s)? \_\_\_\_\_\_\_\_

A5. How many true positives were there (known positive samples that tested positive)?

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

A6. How many true negatives were there (known negative samples that tested negative)?

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

A7. How many false positives were there (known negative samples that tested positive)?

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

A8. How many false negatives were there (known positive samples that tested negative)?

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

A9. Total number of samples

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

B. Repeatability of a qualitative assay

 B1. Were replicates of control or reference samples performed (within-run repeats)?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 B2. How many replicates of control or reference samples were performed? \_\_\_\_\_

 B3. What is the percent concordance for within-run repeats? \_\_\_\_\_

B4. Were samples tested repeatedly between runs?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

B5. How many between-run repeats were performed? \_\_\_\_\_

B6. What is the percent concordance for between-run repeats? \_\_\_\_\_

B7. Do you run multiple positive controls?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

B8. How often are positive controls run if there is just one mutation being assayed?

 \_\_ With each assay

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

B9. How often are positive controls run if several mutations are being assayed?

 \_\_ With each assay

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

B10. If positive controls are rotated when several mutations are assayed, how are they rotated?

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

B11. How often are negative controls run?

 \_\_ With each assay

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

C. Limit of detection (lowest amount of analyte that gives an informative result)

C1. What is the lowest % of mutant or variant allele that can be reliably detected in a wild type background? \_\_\_\_\_\_\_\_

D. Interfering substances (substances that, at a given concentration, may lead to erroneous results)

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

 D1. Were interfering substances assessed?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 D2. What biological matrix materials have been tested for interference? \_\_\_\_\_\_\_\_

 D2i. At what concentrations \_\_\_\_\_

E. Please attach as part of your SOP a description of the bioinformatics methods/program that are used to analyze the assay’s data

 E1. Is the bioinformatics methods/program attached?

 \_\_ Yes

 \_\_ No

 \_\_ Unknown

 \_\_ Not Attached

 \_\_ No program used

FOR QUANTITATIVE ASSAYS ONLY

F. Repeatability of a quantitative assay

F1. How many repeats were performed to establish precision? \_\_\_\_\_

 F2. What is the coefficient of variation at or near the lower limit of quantification? \_\_\_\_\_

F3. What is the coefficient of variation at or near the middle of the analytical measurement range?

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

 F4. What is the coefficient of variation at or near the upper limit of quantification?

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

G. Limit of detection (lowest amount of analyte that can reliably be distinguished from background)

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

G1. What is the lowest amount of analyte that can be distinguished from background with 95% confidence?

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

H. Limits of quantification (lowest and highest concentrations of analyte that can be determined with acceptable total error) \_\_\_\_\_\_\_\_

H1. What is the lowest amount of analyte that can be distinguished from background with acceptable total error? \_\_\_\_\_\_\_\_

H2. What is the highest amount of analyte that can be quantified with acceptable total error?

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

H3. Units of concentration \_\_\_\_\_\_\_\_

I. Reference range

I1. How many samples were used to establish the normal reference range? \_\_\_\_\_

I2. What are the selection criteria for these normal controls? \_\_\_\_\_\_\_\_\_

I3. What is the normal reference range for this assay (with units)? \_\_\_\_\_\_\_\_

J. Reportable range (range of all possible results that are shown to be analytically valid, i.e. with acceptable error)

J1. What is the reportable range of this assay? \_\_\_\_\_\_\_\_

 J1a. Units of reportable range \_\_\_\_\_\_\_\_

**4. Laboratory that is performing this assay**

A. Which type of lab will perform this assay?

 \_\_ Central CLIA Reference Lab

 \_\_ Multiple CLIA-certified Labs

 \_\_ Research Lab(s)

B. Does the lab meet GLP standards?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

C. What is the training and experience of the operator? \_\_\_\_\_\_\_\_

D. Is the lab CLIA certified or accredited? (Only necessary if assay is to be used for medical decision-making)

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

E. Ongoing quality control measures

E1. Is there a program for proficiency testing (PT)?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

 E1a. If yes, how many challenges is PT done? \_\_\_\_\_

 E1b. If yes, how frequently is PT done per year? \_\_\_\_\_

E2. Is there ongoing assessment of technician competency?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known

E3. Is data kept on control samples?

 \_\_ Yes

 \_\_ No

 \_\_ Not Known