Study Chair [ ] LOI/Concept/Protocol #

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**

1 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 the assay and its marker(s) be used in the clinical trial?

○ 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 until specimen fixed/frozen or processed after removal from body) 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
Not Known

F4b If other fixatives are accpetable, 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 next)

G1a Please specify if other type of specimen

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)

G4ai. What is other method for reporting tumor content?

G4b How is tumor content determined?

Flow cytometry
Digital imaging software
Other (Please specify)

G4bi. Other method of determining tumor content?

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)

G5ai. Other method for tumor cell enrichment?

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 commerical product/kit numbers & vendor))

Sanger sequencing
Pyrosequencing
PCR
Mass Spec
Other (Please specify in next field)

A1 If other method is used for mutation assay, please specify

A2 Does the assay use molecular methods to enhance detection of mutations in a heterogeneous specimen?

Yes
No
Not Known

A2a If so, what are the methods?

PNA-clamping
minimal tumor content
Digital PCR
Other (Please specify)

A3 Is assay instrument (sequencer or PCR machine) tested for calibration or performance?

Yes
No
Not Known

A3a If so, please provide the protocols as an Appendix if not included in the SOP

A3b 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

B1e If another reagent, what is it?

C Do you assess DNA Concentration?

Yes
No
Not Known

C1 If you do, how is DNA concentration assessed?

Spectrophotometry
Fluorometry
Other

C1a If other method is used to assess DNA concentration, what is it?

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 (Please specify in next field)

D2a If you assess purity by another technique, what is that method?

D3 Do you assess DNA integrity?

D3a How is DNA integrity assessed?

Amplification of a control
Agilent Bioanalyzer
Bio-Rad 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 SOP's for data analysis.

E1b What software and version is used?

E1c Are there 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)

B8a If Other, please specify frequency

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

With each assay
Other (Please specify)

B9a If other, please specify frequency

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

B10a

B11 How often are negative controls run?

With each assay
Other (Please specify)

B11a If other, please specify frequency

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 willd 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 concentraions

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 progam used

FOR QUANTITATIVE ASSSAYS 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 anlalyte 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 anayte 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 ZLWKXQLWV?

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 the 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 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