**Oncology Pilot Clinical Trial Assay Template:**

**Polymerase Chain Reaction (PCR) Tests**

**For Collection of Performance Validation Data**

**SCOPE:** This template is intended for use by oncology drug sponsors in the Food and Drug Administration’s (FDA) voluntary pilot program described in FDA’s guidance document “[Oncology Drug Products Used with Certain In Vitro Diagnostic Tests: Pilot Program](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/oncology-drug-products-used-certain-in-vitro-diagnostic-tests-pilot-program).” In this and other templates, FDA is providing recommendations regarding data and information that should be submitted to FDA regarding clinical trial assays (CTAs) used in the pivotal clinical trial(s) for oncology drug product(s) for the pilot program. This template is specific for CTAs that use Polymerase Chain Reaction (PCR) technology. Templates for CTAs that use other technologies can be found on [FDA’s website.](https://www.fda.gov/medical-devices/in-vitro-diagnostics/oncology-drug-products-used-certain-in-vitro-diagnostics-pilot-program)

The CTA templates are intended to help oncology drug sponsors and CTA developers collect and provide validation information and performance characteristics for the CTAs, but alternative approaches can be used. To discuss an alternative approach, please contact [OncologyPilotCDRH@fda.hhs.gov](mailto:OncologyPilotCDRH@fda.hhs.gov).

This template reflects FDA’s current thinking on the topic, and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should*, means that something is suggested or recommended, but not required.

As described in FDA’s guidance document, oncology drug sponsors interested in participating in the pilot program should submit a statement of interest to their Investigational New Drug (IND) application, New Drug Applications (NDA), or Biologic License Applications (BLA), as appropriate. The statement of interest should include a statement affirming the oncology drug sponsor’s commitment to provide the information recommended in this template for CTAs used in their pivotal clinical trial(s) and the additional information described in the guidance. Upon receipt of the statement of interest, FDA will follow up with no more than 9 sponsors to request specific information, including the information identified below, to enable FDA to make a decision concerning acceptance into the pilot, based on evaluation of the factors outlined in FDA’s guidance document and provide written feedback that either accepts or rejects the drug product for the pilot program. This template should be used to provide the information recommended in this template only when requested by FDA.

**GENERAL INFORMATION ABOUT THIS TEMPLATE**

When requested by FDA, oncology drug sponsors who have submitted the statement of interest described above should complete the information below, as applicable, using the blue fillable fields. Hot links are provided to navigate to and from sections where data entry into tables is recommended; these data tables are provided in the [Appendix](#Appendix).

Alternatively, oncology drug sponsors have the option of providing the recommended information by submitting documentation from the laboratory that performed the CTA, such as the standard operating procedure (SOP) and a summary of test validation.

1. General Laboratory Information
2. Laboratory name and address: Enter laboratory’s name and address
3. Laboratory contact name and email: Enter person’s name and email address
4. Test name: Enter test name

1. General Test Information
2. Test information

Please provide a general description of the test including:

* 1. Is the test/CTA commercially available?  Yes;  No
     1. if yes, provide the following information:
        1. name of the kit and manufacturer: Enter kit name(s) and manufacturer(s) here
        2. if any modification to the kit were made, describe them: Summarize here
     2. if not, provide the following information:
        1. analyte(s) (e.g., exon 19 deletions and exon 21 (L858) substitution mutations of the *EGFR* gene in DNA): Enter analyte(s)
        2. test method, including specimen type (e.g., reverse transcription, real time PCR in formalin-fixed paraffin-embedded [FFPE] breast tumor tissue): Enter test method including specimen type
  2. CTA components (e.g., probes, reaction mixes, enzymes): Enter CTA components
  3. Extraction method(s): Enter extraction method(s)
  4. List primer sequences used: List PCR primers

1. Number of multiplex reactions? Enter reaction number
2. Instrument/platform used: Enter instrument/platform used
3. Minimum tumor content: Enter amount
4. Minimum nucleic acid amount/range: Enter amount/range
5. Describe the positive control, its use, and what it measures: Positive control
6. Describe the negative control, its use, and what it measures: Negative control
7. Determination of run validity & clinical cut-off
   1. Summarize positive, negative, and internal control criteria that needed to be met before a run was considered valid and individual sample data were analyzed: Summarize here
   2. Briefly summarize the prespecified clinical cut-off (e.g., Ct value) that was used to enroll subjects in the clinical trial: Summarize here

1. Validation Studies
2. Samples used in the validation studies
   1. If surrogate samples (e.g., sample blends, spiked blood) were used, please provide a summary on how they were constructed: Summarize here
   2. If reference samples (e.g., Genome in a bottle) were used, please provide information on their source: Summarize here
   3. Provide numbers of the different sample types used in each validation study in [Table 1](#Table1_sampletypes).
3. Comparator/orthogonal method(s)
4. Please provide information on any comparator and/or orthogonal method(s) used to characterize samples and/or validate the CTA, and the study they were used for in [Table 2](#Comp_Ortho_Table).
5. If laboratory developed test(s) were used:
   1. please provide the laboratory name(s) and address(es): Enter laboratory’s name(s) and address(es)
   2. please provide the stated accuracy of the comparator method(s): Enter accuracy
6. Analytical accuracy - concordance

Analytical accuracy of the CTA is determined relative to a reference method or validated comparator method (orthogonal method). If a comparator method is used, it should have similar panel content and sensitivity to that expected from the CTA (based on the test method and/or previous analytical testing). Well characterized samples should be tested with both the CTA and comparator method.

Please provide the following information:

* 1. Briefly summarize the study design, including the statistical/data analysis methods used to determine analytical concordance: Enter summary
  2. What variant allele frequency frequencies (VAF) were used in this study: Enter VAFs
  3. Positive percent agreement (PPA) and negative percent agreement (NPA) between the CTA and the comparator method should be calculated for the variant(s) and gene(s). Provide concordance summary data in [Table 3](#Table3_AC).

1. Limit of detection (LoD)

Limit of detection (LoD) is determined as the lowest amount of genomic target that the test can consistently detect with stated probability.

Please provide the following information:

1. Briefly summarize the study design, including the statistical/data analysis methods used to determine LoD: Summarize here
2. List how many reagent lots were used in the LoD establishment and/or confirmation study: Enter number
3. Provide a summary of results in [Table 4](#Table3_LoD).
4. Precision

Precision studies are performed to evaluate sources of variation in the test procedure to determine the repeatability and reproducibility of the test.

Please provide the following information:

* + - 1. A brief study design (e.g., full factorial): Summarize here
      2. Runs: Enter number
      3. Days: Enter number
      4. Instruments: Enter number
      5. Reagent lots: Enter number
      6. Operators: Enter number
      7. Number of replicates tested per sample: Enter number
      8. Evaluated at multiple sites?  Yes;  No

1. Was the precision study conducted with the end-to-end workflow (i.e., starting from nucleic acid extraction from tissue or plasma to determining results)?  Yes;  No
2. If only part of the workflow was included in the precision study, specify what was included: Part of the workflow included in the precision study
3. If only part of the workflow was included in the precision study, provide a rationale: Summarize rationale here
4. Summarize statistical/data analysis methods used to determine precision of the CTA: Summarize data analysis here
5. Provide a summary of PPA and NPA for representative variant(s) in gene(s) and for test-wide precision, if applicable, at the variant-, variant type-, and sample-level in [Table 5](#Table5_Precision).
6. Interfering substances

Interfering substances studies evaluate the effects of potentially interfering endogenous and exogenous substances on test performance.

Please provide the following information:

* 1. Briefly summarize the study design, including the statistical/data analysis methods used to evaluate interference: Summarize here

1. Provide a summary of results for each interfering substance tested in [Table 6](#Table6).
2. Exclusivity/cross-reactivity

Exclusivity/cross-reactivity studies evaluate the specificity of the primers or probes used to target specific genes/target regions.

Please provide the following information:

1. Briefly describe the exclusivity/cross-reactivity study performed: Provide description here
2. Provide a summary of cross-reactivity with non-target regions: Provide summary here
3. Stability

Stability studies are conducted to support storage conditions, including the duration of storage, for specimens and stored intermediate products, as applicable.

Please state the stability for:

1. Primary specimens: Enter condition(s) and duration(s)
2. Intermediate specimen products (e.g., cDNA): Enter condition(s) and duration(s)
3. PCR liquid biopsy (LBx) specific information

If the CTA uses LBx, please provide the following information:

1. Please describe any quality measures for the circulating tumor DNA (ctDNA) samples (e.g., fragment analysis): Provide description
2. Limit of blank (LoB) is determined as the highest measurement result that is likely to be observed (with stated probability) for a blank sample (e.g., analyte-negative). If LoB has been performed for your CTA, please describe the study to establish the CTA’s LoB: Provide description and LoB
3. For blood-based tests, short draws and unique components derived from the blood collection process could contribute to interference. Please describe how such interferents were assessed using [Table 6](#Table6).

**Appendix: Data Tables**

**Table 1:** Number of independent samples used for each validation study

|  |  |  |  |
| --- | --- | --- | --- |
| Studies | Clinical | Reference | Surrogate |
| Analytical accuracy - concordance | Enter data | Enter data | Enter data |
| Limit of detection | Enter data | Enter data | Enter data |
| Precision | Enter data | Enter data | Enter data |
| Interfering substances | Enter data | Enter data | Enter data |

To return to “Samples used in the validation studies” click [here](#Samples_used_PCR)

**Table 2:**Comparator/orthogonal method(s)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| For sample | selection or characterization |  | |  | |  | |  |
| Orthogonal method | Information | Analytical accuracy - concordance | | Limit of detection | | Precision | | Interfering substances |
| Name | Enter information |  | |  | |  | |  |
| Technology | Enter information | Yes;  No | | Yes;  No | | Yes;  No | | Yes;  No |
| Performed in-house | Yes;  No |  | |  | |  | |  |
| For test | /CTA validation |  | |  | |  | |  |
| Comparator method  (If different from the orthogonal method) | Information |  | | Analytical accuracy | | - concordance | |  |
| Name | Enter information |  |  | |  | |  | | |
| Technology | Enter information |  | Yes | | No | |  | | |
| Performed in-house | Yes;  No |  |  | |  | |  | | |

To return to “Comparator/orthogonal method(s)” click [here](#Assays)

**Table 3:** Summary of analytical concordance between CTA PCR (CTA) and comparator (Comp) for the variant(s) and gene(s) evaluated for patient enrollment

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene, variant and variant type | Sample type | CTA (+), Comp (+) | CTA (+), Comp (-) | CTA (-), Comp (+) | CTA (-), Comp (-) | Possible variants (n) | Sample (n) | PPA  (% CI\*) | NPA  (% CI\*) |
| Enter gene (e.g., *EGFR*), variant (e.g., T790M), variant type (e.g., SNV | Enter information | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data |
| Enter gene (e.g., *EGFR*), variant (e.g., exon 19, variant type (e.g., deletion) | Enter information | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data |
| Enter gene (e.g., *ALK*), variant (e.g., *NPM1*-*ALK*), variant type (e.g., fusion) | Enter information | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data |

\* CI: Confidence Interval

To return to “Analytical accuracy – concordance” click [here](#Correlation_Comparator_Accuracy)

**Table 4:** Call/detection/hit rate for LoD

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample ID | Gene, variant and variant type | Measurand levels (list examples) | Level selected for LoD (Y/N) | Level (e.g., VAF, CNV, tumor content) | Replicates called (n)/total replicates (n) (%) |
|  | Enter gene (e.g., *EGFR*) | Enter high level | Yes;  No | Enter data | Enter data |
| Enter information | Enter variant (e.g., T790M) | Enter medium level | Yes;  No | Enter data | Enter data |
|  | Enter variant type (e.g., SNV) | Enter low level | Yes;  No | Enter data | Enter data |
|  | Enter gene (e.g., *ALK*) | Enter high level | Yes;  No | Enter data | Enter data |
| Enter information | Enter variant (e.g., *NPM1*-*ALK*),) | Enter medium level | Yes;  No | Enter data | Enter data |
|  | Enter variant type (e.g., fusion) | Enter low level | Yes;  No | Enter data | Enter data |

To return to “Limit of detection” click [here](#LoD)

**Table 5:** Variant PPA/NPA summary for precision

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample ID | Alteration (i.e., gene, variant) | Level (e.g., VAF, tumor content, copy numbers) | Fold LoD (e.g., 1xLoD, 3xLoD) | Number positive/number expected | PPA (% CI) | NPA (% CI) |
| Enter information | Enter gene1 and SNV1 variant (e.g., *EGFR* T790M) | Enter data | Enter data | Enter data | Enter data | Enter data |
| Enter information | Enter gene2 and SNV2 variant (e.g., *EGFR* L858R) | Enter data | Enter data | Enter data | Enter data | Enter data |
| Enter information | Enter gene3 and SNV3 variant (e.g., *EGFR* Exon 19 Del, E746\_A750del) | Enter data | Enter data | Enter data | Enter data | Enter data |

To return to “Precision” click [here](#Precision)

**Table 6:** Summary results for interfering substances study

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Substances | Substance level tested | LoD levels used for variant allele frequency/copy number/tumor content, etc. | # Samples | Replicates/  sample | Failure rate\* | Detection rate\*\* | Call rate (% CI) |
| Enter information (e.g., no interferent) | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data |
| Enter information (e.g., hemoglobin) | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data | Enter data |

\* # of replicate sample failures/# of replicate samples tested; \*\* # of variant detected/#of variants expected

To return to “Interfering substances” click [here](#InterferingSubstances)