NEW YORK STATE | Department of Health | Wadsworth Center

ASSAY APPROVAL IN ONCOLOGY

Please submit the required sections of this form and any necessary documentation.

Submit one hard copy of the entire package and one electronic copy (as a PDF file on a CD or flash drive) to: **Clinical Laboratory Evaluation Program, Wadsworth Center, New York State Department of Health, P.O. Box 509, Empire State Plaza, Albany, NY 12201-0509; Attn: Assay Validation Review.**

Materials submitted, including related data packages, cannot be returned to the laboratory. All materials are maintained under strict confidentiality. As relates to New York State's Freedom of Information Law (commonly called FOIL): The Department's Records Access Officer has advised Wadsworth Center that if documents are marked "proprietary"; "confidential"; or with any labeling indicative of the submitter's desire for an increased level of protection based on the submission content, such protection from immediate release based on a FOIL request is justified. Laboratories will be given an opportunity to block information release if a request for the material is filed under the FOIL, by presenting evidence that the materials contain trade secrets. Marking should minimally appear on the cover page of each unit of material. Documents not marked with such terms will not block release of the submission through a FOIL request. Please refer to New York State Standards Specialty Standards of Practice for Oncology in preparing your submissions.

SECTION 1: GENERAL INFORMATION

Laboratory Name: NYS PFI:

Contact Person:

Phone: Fax: Contact E-mail:

Assay (Test) Name (e.g. KRAS mutation detection)*:

Methodology (e.g., PCR; sequencing; FISH**) If multiple techniques are utilized please list them all:

Analyte(s) included (if different from Assay Name):

Validated Specimen Type(s)

Clinical Purpose:

Laboratory Director/Assistant Director (NYS Certificate of Qualification or Holder for Oncology-Molecular)

CQ Code Signature _____

*If the test comprises a number of individual assays that are combined into a panel and interpreted as such you must clearly describe the composition and application of the panel. Complete packages for each individual assay must be submitted. In addition to validation of each individual assay, the combined panel must also be validated in its combination. For example, if the end result consists of a risk/prediction factor, score or similar, it is this value that must be shown to be accurate, precise and reproducible, and meet all the other criteria described in the general requirements for assay validation.

**For FISH assays to be offered under the Oncology category, special conditions apply. Furthermore, validations must follow the guidelines described for Cytogenetics.

SECTION 2: COMPLETE THIS PART ONLY FOR THOSE SUBMISSION TYPES LISTED BELOW. ALL OTHER SUBMISSIONS REQUIRE A COMPLETE PACKAGE AS DESCRIBED IN SECTION 3

☐ Addition of an assay under an approved exemption: provide the Project ID from your original exemption approval letter, a description of the assay to be added, and attach a summary of the validation performed, and sample reports for all possible outcomes.

☐ Modified FDA or CLEP approved assay: indicate the modification below and attach a summary of the study performed to validate the modification.

☐ Specimen Type ☐ Target Population ☐ Purpose of Testing ☐ Analysis (e.g., qualitative vs. quantitative)

SECTION 3: FOR ALL TESTS EXCEPT THOSE IN SECTION 2, COMPLETE THIS ENTIRE SECTION AND PROVIDE ALL REQUIRED ATTACHMENTS

Please submit the following information, organized with an index or table of contents, as numbered or tabbed attachments as indicated below. If an item is not included, indicate the reason. Indicate the **page numbers and/or tabs where** the items and/or attachments can be found. **SUBMISSIONS THAT ARE NOT ORGANIZED AS DESCRIBED MAY BE RETURNED AND THE REVIEW SIGNIFICANTLY DELAYED.**

SECTION 3.1: STANDARD OPERATING PROCEDURE MANUAL (SOPM)

Procedure manuals must contain all required elements as described in the NYS General Systems Standards, Operating Procedures Sustaining Standard of Practice 2 (SOPM S2) Content (a-q). For FISH and aCGH assays, please adhere to the appropriate guidelines for Cytogenetics.

This is the "cookbook". Everything required to actually perform the procedure should be described here, starting from sample acquisition to data interpretation.

Page/Tab	
	INTRODUCTION:
	Description of the test and its background and/or principle of the assay.
	Description of the genetic structure of gene(s) to be tested. A figure or diagram is most helpful and encouraged; indicating probe or primer binding sites on this diagram is recommended.
	Indications for testing
	Probable clinical relevance of testing for the gene(s) of interest
	References to literature. Submit full reprints of all relevant publications as attachment 3.4 and, if appropriate, databases used for interpretation.
	SPECIMEN REQUIREMENTS:
	Specimen type(s), including minimum volumes/amounts to perform the assay
	Specimen collection, stability and handling
	Specimen rejection criteria (e.g. minimum percent tumor content, DNA quality and quantity metrics)
	DETAILED DESCRIPTION OF THE TEST PROCEDURE, including:

	List of materials required, including essential equipment, reagents (w/ sources), supplies
	Primer/probe sequences and anticipated product size(s) (a table works best)
	Reagent preparation and QC
	Step by step protocol(s)
	PCR cycling conditions
	Indicate whether samples are tested in replicate; if so how many and provide criteria for concordance, i.e. what is the acceptable variation between replicates.
	ASSAY CONTROLS, describe:
	Positive controls: include in each run to verify that the assay works as designed. For quantitative assays at a minimum one high and one low positive control must be included. For assays with an extraction phase, it is recommended to include an extraction control. However, under certain circumstances an internal control (e.g. amplification of a reference gene) may substitute for the extraction control.
	Negative controls: include in each run to verify that the assay does not give false positives.
	Sensitivity controls: it is recommended to mix positive control material into a background of negative control material (e.g. Cells, DNA or RNA), e.g. for 5% sensitivity mix 5 ng positive DNA with 95 ng negative DNA before analysis.
	Specificity controls: to demonstrate that the assay is specific for the intended target/analyte. Especially important for RT-PCR, where a control sample without RT needs to be run to show that amplification products are derived from RNA and not from DNA contamination.
	PCR reagent control: also called no template control
	Amplification control gene: Also called the internal control to verify that absence of a positive signal is not caused by poor quality starting material, e.g. DNA/RNA that cannot be amplified
	Calibration (Standard) curve
	EXPECTED RESULTS, including: **Note**: for PCR, restriction digest, etc., a description of the expected product or band size/pattern is required, e.g. what size bands (or size range) are expected from normal or disease samples. If using quantitative measures, what are the expected or accepted ranges for the result, e.g. C_T values in qPCR. If using melting curve analysis, what is the expected Tm and acceptable range.
	Negative results: include clear criteria for calling a result negative; or if appropriate, what quantitative range does the result have to fall in to be negative.
	Positive results: include clear criteria for calling a result positive; or if appropriate, what quantitative range does the result have to fall in to be positive.
	Calculations & Algorithms used to analyze the data
	Assay acceptance criteria: identify the critical steps in the test procedure and the quality control measures taken to control and monitor assay performance for consistent and reliable results.
	Rejection criteria

	Troubleshooting
	Assay interferences and limitations

SECTION 3.2: REQUISITION, REPORTING, & ADVERTISING MATERIALS

Sample reports need to be submitted on the actual report form that is sent to physicians and include all information that would be sent to the ordering physician. This includes the actual result, the testing methodology used and the limits of sensitivity (both technical and diagnostic) of the method, an interpretation of the results, compliant with **Reporting Sustaining Standard of Practice 1 (Reporting S1): Report Content** and any relevant disclaimers required by the federal government such as that required for an Analyte Specific Reagent (ASR).

Page/Tab

	Patient reports (positive, negative, inconclusive)
	Patient requisition forms compliant with **Requisition Sustaining Standard of Practice 4 (Requisition S4): Request Form**
	Advertising material aimed at physicians and advertising material aimed at the general public

SECTION 3.3: VALIDATION SUMMARY, PROTOCOL AND REPRESENTATIVE DATA

This should include a summary that demonstrates how the analytical and clinical performance characteristics of the assay were established. **However, do not submit test development data** (e.g. comparison of different conditions or reagents used in the development of the final submitted procedure). In general, for validation of accuracy, studies must include a minimum of 10 positive and 10 negative patient/clinical samples, except in cases where positive patient/clinical samples are difficult to obtain. Please include actual instrument printouts, worksheets, or charts from a representative run, including all calibration and quality control materials, and include all raw data for positive samples. Tables summarizing the results of all validation experiments should also be provided. Experience has shown that it is generally difficult, if not impossible, to evaluate photocopies of runs/results. Therefore we require that you submit originals (e.g. gel pictures, real-time PCR printouts, chromatographs, etc) or high quality reproductions that are **clearly labeled** and in color when appropriate. All validations must be performed at the actual testing site. For the accuracy studies, samples preferably are split with another lab, although comparisons within the same lab with a different method (e.g. allele specific PCR vs. sequencing) may be acceptable. Performance characteristics must be established for all specimen matrices. For FISH and aCGH assays, please adhere to the appropriate guidelines for Cytogenetics. Confirmation of aCGH assays may be performed using PCR in place of FISH, if preferred. Complete and submit this worksheet with your application:

a) No. of samples tested: total positive negative

Accuracy (reference method and/or laboratory)

Concordance

b) Reportable (calibration) range

c) Precision (reproducibility) of results: Note: a minimum of 3 negative and 3 positive patient/clinical samples should be assayed in at least triplicate (intra-assay) and three separate runs (inter-assay) to establish precision/reproducibility.

Intra-run: No. samples: No. repeats: No. runs:

%CV: high medium low negative

Concordance, if qualitative

Inter-run: No. samples: No. repeats: No. runs:

%CV: high medium low negative

Concordance, if qualitative

d) For quantitative (RT)-PCR, objective criteria (ranges) need to be established that define acceptability of results.

- Slope of Standard curve(s)
- R^2 of Standard curve(s)
- If one standard curve for multiple genes, is amplification efficiency equal?

e) Sensitivity and Specificity

- Analytic sensitivity: given the technical parameters of the assay, what is the minimal amount of target, e.g. malignant cells, that can be detected?
- Analytic specificity
- Clinical sensitivity
- Clinical specificity

f) Clinical limitations: this may require a literature search. While an exact number is not always available, this should describe how many true positive cases of the disease are expected to be detected at the stated analytical sensitivity. It is also recommended to include a statement of how many true cases may be missed on the patient report, which for the physician may be more helpful.

SECTION 3.4: REFERENCES & PRODUCT INSERTS

Page/Tab	
	Copies of literature references that describe the scientific basis and support the clinical validity of the assay.
	Test kit package insert if the test is commercially distributed, or package inserts for any commercially prepared reagents.