NEW YORK STATE | Department of Health | Wadsworth Center

FISH ASSAY APPROVAL IN CYTOGENETICS

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: **The 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.

SECTION 1: GENERAL INFORMATION

Laboratory Name:_____NYS PFI: _____

Contact Person:_____

Phone:_____Fax: _____Contact E-mail: _____

Assay (Test) Name (e.g. multiple myeloma panel): _____

Methodology (metaphase, interphase, or both):_____

Analyte(s) included; list all FISH probes:

_____

_____

Validated Specimen Type(s):_____

Clinical Purpose:_____

_____

Laboratory Director or responsible Assistant Director (CQ holder) for Cytogenetics:

CQ Code_____Signature _____

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

☐ FDA approved assays: Provide the information described in Section 3.5, if applicable, and Section 3.6.

FDA PMA/510(k) number:_____

☐ Modified FDA or NYS approved assay: Describe the modification or change and provide a summary of the validation study and results.

FDA PMA/510(k) number:_____

☐ Specimen type ☐ Target population ☐ Intended Use ☐ Other

☐ Addition of a FISH assay under an approved exemption: Validation must include all applicable elements described in these guidelines. Submit the Project ID number under which the Core Exemption was granted, a description of the assay to be added, and a summary of the validation performed (including specimen type, cell stage, and validation results).

Core Exemption PID number:_____

SECTION 3: COMPLETE THIS ENTIRE SECTION AND PROVIDE ALL REQUIRED ATTACHMENTS

Please submit the following information, organized as numbered or tabbed attachments as indicated below. If an item is not included, explain why this is so. 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.** Please refer to the New York State Cytogenetics Specialty Standards of Practice in preparing your submissions. Laboratories may be granted an exemption from submission of full method validation material for FISH following demonstration of a satisfactory validation process. An exemption request may be included with the validation material.

Section 3.1: OVERVIEW

Page/Tab	
	Brief description of the disease and the test
	Brief description of the affected gene(s) or region(s) and the nature and clinical relevance of the aberration(s)
	Indications for testing/reason for referral
	Bibliography of pertinent references. Include hard copies of references for uncommon tests.
	Specimen type(s) to be tested (cultured/uncultured, fixed/fresh); specify tumor types.
	Cell stage(s) to be tested
	Probe target, chromosomal band location, vendor, catalog number, and package insert

Section 3.2: SPECIMEN AND REQUISITION REQUIREMENTS

Page/Tab	
	Specimen collection and shipping requirements
	Specimen rejection criteria
	Sample requisition form, compliant with Requisition Sustaining Standard of Practice 4 (Requisition S4): Request Form.

Section 3.3: MATERIALS AND METHODS

This section should include all information relevant to the test. Procedures included in previously approved tests do not need to be re-submitted unless altered, but please include your laboratory's document identifier and the CLEP PID number of the approval for any SOPs relevant to the current test.

Page/Tab	
	Reagent preparation and QC
	Vendor and catalog number for all supplies, reagents, equipment, and instrumentation
	Step by step protocol. Refer to Operating Procedures Sustaining Standard of Practice 2 (SOPM S2): Content.
	Describe controls, how they are verified, and how they are used
	For prenatal testing, set target turnaround time and describe how this is achieved and monitored
	Criteria to accept and reject results for an individual specimen and for a run, as appropriate
	Technical limitations and troubleshooting guide
	The laboratory's procedures for reporting results following completion of testing
	Safety issues
	Expected results. For example, describe reportable signal patterns.
	Cell stage and number of cells to be examined
	Cell selection criteria
	Quality assurance, including biannual performance verification for each FISH test. Refer to Quality Assessment Sustaining Standard of Practice 3 (QA S3): Ongoing Verification of Examination Accuracy.
	For FISH panels that combine results of multiple probes using an algorithm, describe the algorithm and how it is applied.

Section 3.4: VALIDATION

It is important that the laboratory accurately and completely convey the validation process, its results, and criteria for acceptability of these results. Do not submit test development data (e.g. comparison of different conditions used in developing the final procedures). Ensure that any submitted images are high-quality and appropriately labeled. The metaphase validation components of a test to be used only in non-dividing cells may be performed using an appropriate mitotic specimen type. Results from a given slide may be used to satisfy more than one validation requirement where appropriate. The requirements below apply in most circumstances to FISH testing for both constitutional and acquired aberrations.

Page/Tab	
	<u>Validation overview</u> Submit: Narrative describing validation studies and criteria for clearing a new test for diagnostic use. Describe each specimen used in the validation, including specimen type, cell stage, FISH results, chromosome analysis (if performed) and clinical features. Describe criteria used to confirm accuracy. If multiple assays or probes are to be used as a panel, describe the panel and any analysis algorithm.
	<u>Confirm correct probe chromosome band location</u> Requirement: Document location in five metaphase cells from a male specimen of any specimen type using reverse DAPI or sequential G-banding and FISH. Required for all FISH assays, including those that will be used clinically only in interphase cells. Submit: One sample image of reverse-DAPI or sequential G-banding and FISH. Label each chromosome with its chromosome number and indicate the FISH target region.

	Probe sensitivity and probe specificity Requirements: Demonstrate that each probe hybridizes to its target and nowhere else. Use a minimum of five samples from five normal males. Typically based on analysis of 40 metaphase chromosomes bearing the target locus. Validation of autosomal loci generally will require analysis of 20 metaphase cells. Validation of sex chromosome loci generally will require 40 metaphase cells. Minimum sensitivity should be 95% and minimum specificity should be 98%. Validation samples should include all specimen types intended for clinical testing. Required for all FISH assays, including those that will be used clinically only in interphase cells. Submit: For each specimen, the specimen type, the test results, and the data used to document that the specimens are from normal individuals. Include probe sensitivity and probe specificity, expressed as the percentage of cells analyzed.
	Accuracy Requirements: Compare FISH results at the target site(s) to results of G-banded chromosome analysis or other second method or results from a second laboratory. For tests that rely on cutoff values determined by the laboratory (typically, normal range cutoffs for leukemia/lymphoma), use three normal and three abnormal specimens. For tests that rely on values derived from the literature (typically, ratio of signals from two or more probes in solid tumors), use a minimum of 10 normal and 10 abnormal samples. If the test is for a rare aberration and sufficient abnormal specimens cannot be obtained, document this fact. Apply any scoring algorithms incorporating multiple FISH tests or probes to demonstrate accuracy of the algorithms; include all tumor types to be tested, if applicable. Submit: Summarize accuracy data and include FISH images for at least three normal and three abnormal specimens. A table works well for presenting the data.
	Normal range for interphase FISH assays Requirements: Determine the normal range using a sufficient number of specimens and nuclei to establish confidence limits. This requirement applies to testing for both constitutional and acquired aberrations in interphase cells. Typically, a minimum of 20 normal specimens, 200 cells per specimen are required for cancer testing. Normal cut-off values must be calculated separately for each reportable signal pattern in each specimen or tissue type (e.g. cutoff values to be used for blood specimens should be based on blood specimen data). Generally not required for tests that are based on a ratio of number of signals for two probes in solid tumors. Normal range cutoffs may be calculated using methods such as the BETAINV function or a multiple of the standard deviation. Submit: FISH results for each sample for each reportable signal pattern. Normal cut-off values for reportable aberrations specific to specimen type, tissue type, and signal pattern.
	Reproducibility Requirements: For interphase FISH assays to be used for cancer testing, demonstrate intra- and inter-assay reproducibility. Typically, intra-assay reproducibility is based on results of at least three samples tested in triplicate on one day; inter-assay reproducibility is based on results of two additional replicates of each sample, each replicate tested on a different day. While it is expected that results for replicates may differ quantitatively, the results/interpretation should be qualitatively similar. Submit: Describe numbers of samples, replicates, and runs used. Present results for each replicate as percent normal or abnormal cells or as the ratio of signal numbers for the probes. A table works well for presenting the data.
	Clinical validity Submit: A brief summary of the clinical sensitivity and clinical specificity of the test and how they were established. Include pertinent literature references and/or clinical study data that support the laboratory's intended use of the test.

Section 3.5: INFORMED CONSENT

Cytogenetic testing for constitutional disorders requires informed consent by the patient or legal guardian consistent with Civil Rights Law Article 7, Section 79-l. The laboratory should have a consent form and policy in place for ensuring informed consent and provide to clinicians consent materials that include the items below. Consent materials should be specific to the indicated condition; generic descriptions of FISH testing are not sufficient.

Page/Tab	
	A general description of the disease or condition
	Description of the test
	Principle of the test
	Meaning of a positive test result
	Meaning of a negative test result
	Statement regarding test limitations
	Confidentiality of test results and who will receive them
	Statement that further testing or additional physician consults may be warranted
	Statement that genetic counseling be offered *prior to* signing the form and that it be available after the test.
	Statement that any remaining sample will be destroyed after no more than 60 days. Longer retention requires active consent by the patient/guardian (the patient must "opt-in" to longer retention). Be clear about what is being retained (e.g. blood, cell pellet, etc). Consent for longer specimen retention should be separated from consent for the test.
	Signature lines for patient/guardian and healthcare personnel.

Section 3.6: SAMPLE REPORTS

Submit sample reports for normal, abnormal, and inconclusive results and as described below. Sample reports should be appropriately-redacted patient reports or mock reports that include all of the report elements that are found normally in the laboratory's patient reports. Reports must be compliant with Reporting Sustaining Standard of Practice 1 (Reporting S1): Report Content and Cytogenetics Standards 21-22.

Page/Tab	
	Test result using appropriate ISCN nomenclature
	Specific interpretation of results
	Explanation of test limitations and any required disclaimer statements, e.g. ASR disclaimer
	Specimen information (date collected, received, reported, accession no., patient name and date of birth, indication for testing, sample type)
	Signature of Laboratory Director or Assistant Director responsible for the category who reviewed/approved the case.
	Reports should include number of cells analyzed and cutoff values.
	Provide a sample report showing a negative result and sample reports showing positive results for each test outcome (e.g. deletion and duplication). For a FISH panel, a positive result may include normal findings for other probes in the panel.