**UW Medicine - Pathology**

400-08-01-10

Fluorescence in Situ Hybridization (FISH) Validation Procedure

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| Adopted Date: 06/2003Review Date: 09/2005Revision Date: 03/2007 |

PURPOSE

Fluorescence In-Situ Hybridization (FISH) testing using commercial probes must be verified as informative and accurate in this clinical laboratory.

PROCEDURE

1. **Validation Procedures**
	1. Documentation of validation for each probe is kept in the FISH Validation notebooks by probe. Probes should be validated with the tissue type where they will be used. FDA approved probes are not subject to the procedures below except for ongoing monitoring and documentation that performance characteristics are the same or better than stated by the manufacturer in the product insert. Probe check slides are purchased from Vysis for this purpose, which is done once a year.
	2. Reports must include a paragraph stating:  *The FISH test was developed and its performance characteristics determined by the UWMC Cytogenetics Laboratory. Pursuant to the requirements of CLIA 1988, this laboratory has established and verified the test's accuracy and precision. The test has not been cleared or approved by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.*
	3. Reporting should include probe identification and lot, and also number of cells exhibiting normal, abnormal and other configurations. A minimum of two images should be captured for an abnormal case and one image for a normal case.
		1. **Initial validation of tests using known abnormals:** Analytic Specific Reagents (*ASR) tests*: these tests must be validated in the laboratory when first introduced. This validation is done by using the reagent(s) on control positive and negative samples to validate the assays ability to detect the appropriate abnormality. Positive controls used are obtained through: 1) Control positive cell lines from the NIGMS Human Mutant Cell Repository at Coriell, 2) Interlaboratory exchange, e.g., CHRMC slides, 3) CAP FISH PT unknowns, 4) Clinical corroboration of patient specimens.
		2. **Localization/Cross hybridization:**  Score a minimum of 5 banded metaphase cells to verify the probe hybridizes to the target chromosome and no other (cross hybridization). Score each target in a cocktail separately. Keep one or more images of a typical hybridized cell in the Validation notebook.
		3. **Sensitivity/Specificity:** Score a minimum of 200 distinct genomic targets (1 genomic target is one labeled chromatid) and record percentage of metaphases with the expected signal pattern (Sensitivity) and percentage of signals that hybridize to the correct locus and no other loci (Specificity). Score each target in a cocktail separately. Sensitivity and Specificity should be 98% or better with all probes used in the laboratory.
		4. **Ongoing monitoring:** Continuous monitoring of FISH test results for probe contamination or degradation as well as hybridization efficiency and lack of background should be done with each case. Document any variation or unexpected results in the GCS database under each case in the field- Comments: Various. See corrective action below. Subsequent changes of probe lots can be validated by overlapping use of old and new lots.
		5. **Reportable Reference ranges:** After compiling the FISH results for a specific probe from ten normal (2000 nuclei) and abnormal cases, an expected range for the probe in our laboratory is determined. A database with normal and abnormal cases is then used to determine reportable reference ranges for mosaicism at different confidence levels. A positive (abnormal) diagnosis for a probe is set by the BETAINV function in the “Pooledcontrols.xls” file as described in reference 4 and recorded in the ProbeInventory database.
2. **General ScoringCriteria/Exclusion Criteria** (See individual probe protocol for specific scoring criteria).
	1. Probe information: Expected signal loci, fluorophores used, control probes, expected size and brightness of signals should all be reviewed prior to scoring (see probes data sheet). Signals should be bright, punctate or oval-shaped, not diffuse. Scoring must take into account cell morphology: only single, non-overlapped cells with regular outlines should be used. Background should be low enough to not interfere with scoring. If background shows punctate signals of a certain spectra, scoring for that color probe on cells should not be done. Individual target signal color should be evaluated using all filter sets.
	2. Control probes must be used and documented in the FISH analysis where appropriate (see specific probe). Results can be considered valid only if appropriate controls were informative (see individual procedures). If a probe does not identify an internal control, use a control sample (e.g., Y-chromosome probe).
	3. A minimum of two individuals should score IFISH results for each probe/case in a *single blind* scheme. Care must be taken to insure each scorer scores unique cells: either from different slides or different target areas on one slide. If concordance between scorers is <85% then they should discuss their scoring criteria and rescore for the other scorers’ cells. If concordance is still low, a third scorer should look at the same cells. Only results with greater than 90% concordance can be reported. Counts can be extended past 200 if necessary and can be useful in detecting abnormalities present at low levels. Counts can be extended 500-1000 cells/reader if necessary.

### Number of cells analyzed: Table 4

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| Probe or Test | **Cells analyzed** | **Notes** |
| AneuVysion | 100 | By 2 techs minimum |
| Microdeletion | 10 | Increase to 100 for mosaicism |
| Marker | 10 | Increase to 100 for mosaicism |
| Telomere | 10 | Increase to 100 for mosaicism |
| Interphase | 100 | By 2 techs |

### Corrective action

If probe fails to hybridize, prepare new reagents and repeat. If morphology of preparation (interphase or metaphase preparation is inadequate, repeat harvest (hypotonic and fixative may need to be changed). If probe fails to hybridize to appropriate chromosomal site, verify the correct probe was used. Order new probe if necessary and carry out appropriate control (normal/abnormal cell lines). Check microscope and imaging system for appropriate setting.

REFERENCES

1. Standards and Guidelines for Clinical Genetics Laboratories, American College of Medical Genetics, 2005 Edition. <http://www.acmg.net/Pages/ACMG_Activities/stds-2002/e.htm>
2. A Multicenter Investigation With Interphase Fluorescence In Situ Hybridization Using X- and Y-Chromosome Probes, American Journal of Medical Genetics 76:318-326 (1998).
3. A Multicenter Investigation with D-FISH BCR/ABL1 Probes, Cancer Genetics Cytogenetics 116:97-104 (2000).
4. Guidance for Fluorescence *in Situ* Hybridization Testing in Hematologic Disorders, Journal of Molecular Diagnostics, Vol. 9, No. 2, April 2007

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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 Cytogenetics Supervisor