Study Chair: LOI/Concept/Protocol #

DNA-based In situ hybridization biomarker template (FISH, CISH)

This is a template for use in outlining the known status of a FISH or CISH assay that is to be used in a trial. It is intended to be used for assays measuring single genetic variations such as specific translocations, gene amplifications or deletions. It is not intended for array CGH or similar multiplex DNA in situ hybridization assays. Not all parameters may be known a priori. Please enter as much information as you can. Enter N/Afor not available or applicable where appropriate.

It is recommended that Ventura et al., FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J. Mol. Diagn. 8:141-151, 2006 be read as a reference before completing this template.

This template requires detailed information that may be known only by laboratorians, scientists who work in clinical laboratories and should be collaborating closely with clinical trialists. Please be sure to collect the appropriate responses before filling out this form. The template has the following sections with information needed from both trialists and laboratorians:

Section	Heading
1.	Assay, Patient and Specimen Parameters–Trialists and Laboratorians
2 -6.	Probe Characteristics – Laboratorians
7.	Design of In Situ Hybridization Assay - Laboratorians
8.	Assay Performance – Laboratorians
9.	Laboratory Information – Trialists and Laboratorians

1. Assay, Patient and Specimen Parameters

A. Type of DNA In Situ Hybridization Assay

◯ Interphase ◯ Metaphase

B. Type of DNA In Situ Hybridization Probes

◯ Break-apart ◯ Dual Fusion ◯ Other (Please Specify)

B1. Specify Other:

C. Probes

C1. Probe 1
C2. Probe 2
C3. Probe 3
C4. Probe 4
C5. Probe 5

D. How will assay and its marker be used in the clinical trial (Integral, Integrated, or Research)

Integral

◯ **Integral** ◯ **Integrated** ◯ **Research**

Research

E. Assay Purpose

Treatment Assignment

E1. Please specify if other

F. Will assay be provided by a Central Reference CLIA Lab, Multiple CLIA-certified Labs or Research Labs?

◉ **Central Reference CLIA Lab** ◯ **Multiple CLIA Labs** ◯ **Research Labs**

G. Source and Collection of Specimens

G1. Specimen Type

Blood
Bone Marrow
Needle Biopsy
Incisional Biopsy
Excisional Biopsy
FNA

G1a. Please specify if other

G2. Tissue Collection supported in Trial

Voluntary

G3. Genetic syndromes that may impact findings, e.g Trisomy 21 or a disorder that may cause secondary aberrations (Lynch Syndrome)

G3a. Was radiation therapy given

Yes No

G3b. If Radiation therapy was given, what biomarker(s) was used to assess the effect of radiation?

H. Pre-Analytic Variables that may affect assay results

For Blood or bone marrow Specimens

H1. What was specimen collected in?

Heparin
EDTA
Acid-Citrate-Dextrose (ACD)
Other (please specify)

EDTA
Acid-Citrate-Dextrose (ACD)
Other (Please specify)

H1a. Please specify

H1b. Was specimen cultured for metaphase study?

Yes
No
Unknown
Not Applicable

H1bi. How long should specimen be cultured, if cultured?

24 Hours
24 - 48 Hours
Other (Please Specify)

H1bii. Other, specify

If Specimen Not Cultured

H1Ci. Will erythrocytes be lysed with Ammonium Chloride

◯ Yes ◯ No ◯ Unknown ◯ Not Applicable

H1Cii. Will cells be concentrated by density gradient centrifugation

◯ Yes ◯ No ◯ Unknown ◯ Not Applicable

H1Ciii. Will cells be fixed before reacting with probes?

◯ Yes ◯ No ◯ Unknown ◯ Not Applicable

H1Civ. What fixative if used?

Methanol/Acetic Acid
10% Buffered Formalin
Not Applicable
Other (Please Specify)

H1Cv. Please specify

For Tissue Specimens

I1. Type of specimen stabilization

Chemical Fixation
Frozen
Both
Other (Please Specify)

I1ai. Please specify if other

I2. If fixed, what is fixative?

10% Neutral Buffered Formalin
Bouin's
Other

I2a. If other fixative, what was it?

I2b. If fixed, what is the shortest fixation time allowed (Hours)?

I2c. If fixed, what is the longest fixation time allowed (Hours)?

I3. If frozen, how will specimen be frozen?

- Flash Frozen
- Embedded in OCT, then frozen
- Controlled rate cryopreservation

J. Storage of specimen

- -20 Degrees Celsius
- -80 Degrees Celsius
- -100 to -120 Degrees Celsius
- Vapor Phase Liquid Nitrogen
- 4 Degrees Celsius

J1. How long will tissue be stored (please include unit of time, eg days, months)?

J1a. Units of time

- Days
- Weeks
- Months
- Years
- Refused
- Unknown
- Don't Know

K. Specimen Characteristics

K1. Does the specimen consist of whole nuclei or sections of nuclei, eg. Sections of formalin-fixed, paraffin-embedded tissue?

- Whole Nuclei
- Sections of Nuclei

K1a. If sections of tissue, how thick are the sections (in microns)?

K2. What is the minimum number of nuclei counted?

K3. How was that minimum number of nuclei to be analyzed determined to be adequate/representative?

K4. Digestion or other steps to improve probe binding

K5. Is the marker stable when the storage time is:

- < 7 days
- 7 - 30 days
- > 30 days
- Not Known

2. Probe 1 Characteristics

A. Type of probe

Oligonucleotide
BAC Clone
Other (Please Specify)

A1. If other, please specify

B. What is the probe label (FITC, Quantum dots, etc)

Quantum Dots
Alexa Fluor
Other (Please Specify)

B1. If other, please specify

C. Length of probe in nucleotides

D. What is the source of the probe, Commercial or synthesized in-house?

Commercial
Synthesized In-House

D1. If commercial, who was the manufacturer?

D1i. What is the lot number?

E. How was the probe validated?

F. How was specificity of the probe demonstrated?

Normal Metaphase Location
Verification on BAC Clone site (http://genome.ucsc.edu)
Other (Please Specify)

F1a. If other, please specify

G. Has the proper chromosomal location of the probe target been verified by metaphase FISH?

Yes
No
Unknown

H. Was the probe tested on cell lines that have the genetic change?

Yes
No
Unknown

I. Have any cross-reactive or interfering substances been identified that may confound interpretation of the results with this probe?

Yes
No
Unknown

I1. If yes, what are they?

3. Probe 2 Characteristics

A. Type of probe

Oligonucleotide
BAC Clone
Other (Please Specify)

A1. If other, please specify

B. What is the probe label (FITC, Quantum dots, etc)

Quantum Dots
Alexa Fluor
Other (Please Specify)

B1. If other, please specify

C. Length of probe in nucleotides

D. What is the source of the probe, Commercial or synthesized in-house?

Commercial
Synthesized In-House

D1. If commercial, who was the manufacturer?

D1i. What is the lot number?

E. How was the probe validated?

F. How was specificity of the probe demonstrated?

Normal Metaphase Location
Verification on BAC Clone site (http://genome.ucsc.edu)
Other (Please Specify)

F1a. If other, please specify

G. Has the proper chromosomal location of the probe target been verified by metaphase FISH?

Yes
No
Unknown

H. Was the probe tested on cell lines that have the genetic change?

Yes
No
Unknown

I. Have any cross-reactive or interfering substances been identified that may confound interpretation of the results with this probe?

Yes
No
Unknown

I1. If yes, what are they?

4. Probe 3 Characteristics

A. Type of probe

Oligonucleotide
BAC Clone
Other (Please Specify)

A1. If other, please specify

B. What is the probe label (FITC, Quantum dots, etc)

Quantum Dots
Alexa Fluor
Other (Please Specify)

B1. If other, please specify

C. Length of probe in nt

D. What is the source of the probe, Commercial or synthesized in-house?

Commercial
Synthesized In-House

D1. If commercial, who was the manufacturer?

D1i. What is the lot number?

E. How was the probe validated?

F. How was specificity of the probe demonstrated?

Normal Metaphase Location
Verification on BAC Clone site (http://genome.ucsc.edu)
Other (Please Specify)

F1a. If other, please specify

G. Has the proper chromosomal location of the probe target been verified by metaphase FISH?

Yes
No
Unknown

H. Was the probe tested on cell lines that have the genetic change?

Yes
No
Unknown

I. Have any cross-reactive or interfering substances been identified that may confound interpretation of the results with this probe?

Yes
No
Unknown

I1. If yes, what are they?

5. Probe 4 Characteristics

A. Type of probe

Oligonucleotide
BAC Clone
Other (Please Specify)

A1. If other, please specify

B. What is the probe label (FITC, Quantum dots, etc)

Quantum Dots
Alexa Fluor
Other (Please Specify)

B1. If other, please specify

C. Length of probe in nt

D. What is the source of the probe, Commercial or synthesized in-house?

Commercial
Synthesized In-House

D1. If commercial, who was the manufacturer?

D1i. What is the lot number?

E. How was the probe validated?

F. How was specificity of the probe demonstrated?

Normal Metaphase Location
Verification on BAC Clone site (http://genome.ucsc.edu)
Other (Please Specify)

F1a. If other, please specify

G. Has the proper chromosomal location of the probe target been verified by metaphase FISH?

Yes
No
Unknown

H. Was the probe tested on cell lines that have the genetic change?

Yes
No
Unknown

I. Have any cross-reactive or interfering substances been identified that may confound interpretation of the results with this probe?

Yes
No
Unknown

I1. If yes, what are they?

6. Probe 5 Characteristics

A. Type of probe

Oligonucleotide
BAC Clone
Other (Please Specify)

A1. If other, please specify

B. What is the probe label (FITC, Quantum dots, etc)

FITC
Quantum Dots
Alexa Fluor
Other (Please Specify)

B1. If other, please specify

C. Length of probe in nt

D. What is the source of the probe, Commercial or synthesized in-house?

Commercial
Synthesized In-House

D1. If commercial, who was the manufacturer?

D1i. What is the lot number?

E. How was the probe validated?

F. How was specificity of the probe demonstrated?

Normal Metaphase Location
Verification on BAC Clone site (http://genome.ucsc.edu)
Other (Please Specify)

F1a. If other, please specify

G. Has the proper chromosomal location of the probe target been verified by metaphase FISH?

Yes
No
Unknown

H. Was the probe tested on cell lines that have the genetic change?

Yes
No
Unknown

Yes
No
Unknown

I. Have any cross-reactive or interfering substances been identified that may confound interpretation of the results with this probe?

Yes
No
Unknown

Yes
No
Unknown

I1. If yes, what are they?

7. Design of In Situ Hybridization Assay

A. Assay Design

A1. Describe the platform of the assay

A1a. Platform

A1b. Model Number

A1c. UDI (Unique Device Identifier - supplied on lab equipment)
http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/UniqueDeviceIdentifiers/default.htm

A1d. Is the platform cleared by FDA

Yes
No
Unknown

A2. Is there an SOP for the assay

Yes
No
Unknown

A2a. If there is a SOP, is it attached as an appendix?

Yes
No
Unknown

B. Type of In Situ Hybridization

FISH
CISH
Other (Please Specify)

B1. If other, please specify

B2. Assay method (e.g., direct, indirect, other)

Direct
Indirect
Other

Direct
Indirect
Other

B2a. Please specify

C. Details of positive and negative controls for the assay

C1. Positive control for Probe 1

C1. Negative control for Probe 1

C2. Positive control for Probe 2

C2. Negative control for Probe 2

C3. Positive control for Probe 3

C3. Negative control for Probe 3

C4. Positive control for Probe 4

C4. Negative control for Probe 4

C5. Positive control for Probe 5

C5. Negative control for Probe 5

8. Assay Performance

A. Assistance with Interpretation

A1. Will a pathologist assist with selection of the part of the specimen to be analyzed?

Yes
No
Unknown

A2. Will a cytogeneticist assist with the interpretation of the FISH patterns/results vs. the genetic/chromosomal mechanisms and/or artifacts of processing/cell overlaps that can confound the FISH results?

Yes
No
Unknown

B. What statistical test(s) were used to validate the assay results?

C. How was a clinically relevant threshold selected?
- Literature
- Pilot Clinical Study
- Medical Practice Guidelines
- Non-clinical Data (e.g., cell line)
- Other

C1. If Other, please define

D. Will quantitative data be collected?
- Yes
- No
- Unknown
- Not Applicable

E. Will data be presented qualitatively?
- Yes
- No
- Unknown
- Not Applicable

F. If qualitative data provided, how will thresholds be determined, eg Positive vs Negative?

G. What is the threshold or cut-off?

H. How is/was the threshold/cutoff value validated before using the assay in this trial?

I. Were assay conditions standardized to minimize variance, e.g. automated tissue processors and/or stainers?
- Yes
- No
- Unknown

I1. If yes, what tissue processor/stainer was used?

J. Reproducibility of assay

J1. How was hybridization quality assessed?

J2. Were replicates done?
- Yes
- No

J2a. How many replicates were done?

J3. What is the intra-lab reproducibility (%CV)

J4. What is the inter-lab reproducibility (same specimens)?

J5. Are there at least 2 readers for each sample?

- Yes
- No
- Unknown

J5a. If so what is the agreement between readers?

J5b. How are differences between readers resolved?

- Different Runs of the same assay
- Different Runs of another assay of the same technolo
- Different Runs of another assay of a different technolc
- Different reading by the same reader or instrument
- Different reading by a different reader or instrument
- Panel or arbitration
- Other, Specify

J5bi. If other, please specify

K. Assay discrimination

K1. How will staining artifacts be identified and handled (especially if image analysis is used)?

K2. If image analysis is used, describe how stacks will be analyzed to check for artifacts

K3. How will tumor heterogeneity be handled?

L. Details regarding the quantitative component of the assay

L1. What strategy will be used to select the fields to be analyzed?

L2. How many normal controls will be used to establish a false-positive cutoff for a given probe?

L2a. What will be the selection criteria for these normal controls?

L2b. How will the cells of interest be distinguished from other cells?

L2c. Was reference material used to generate this cutoff?

- Yes
- No
- Unknown

L2d. Has the assay been cleared by the FDA?

- Yes
- No
- Unknown

L2e. What is the accuracy for detecting alterations in the target?

9. Laboratory

A. Does the lab meet GLP standards?

Yes
No
Unknown

Good Laboratory Practices (GLP) are defined by the FDA in their guidance at:
http://www.fda.gov/downloads/ICECI/EnforcementActions/BioresearchMonitoring/ucm133730.pdf

B. What is the training and experience of the laboratory staff?