**UW Medicine - Pathology**

400-04-01-04

Fluorescence in Situ Hybridization (FISH) - Detection of Numerical, Microdeletions, Translocations or rearrangements, and Amplification abnormalities

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| Adopted Date: 3/4/2013  Review Date: 4/10/2013  Revision Date: |

PURPOSE

Fluorescence In-Situ Hybridization (FISH) is a molecular test used to quickly identify specific chromosome abnormalities involving specific genes or regions. Abnormalities detected by FISH include deletions, duplications, translocations or rearrangements, amplification and chromosome or gene copy number.

PROCEDURE

### Material and Equipment

* + - 1. Water bath
      2. Magnetic stirrer
      3. Centrifuge
      4. Vortex mixer
      5. Incubator
      6. Coplin jars
      7. Thermometer
      8. pH meter
      9. Paper cement
      10. Fluorescence microscope equipped with recommended filters
      11. Coverslips
      12. Pipettes
      13. Microfuge tubes
      14. Timer
      15. Forceps
      16. Diamond pen

### Reagents, Probes and Solutions

### Probes

a. Abbott probes: Store at -10 to -30C.

b. Cytocell probes: Store at -10 to -30C.

c. Dako probes: Store at 2 to 8C.

d. DAPI II (Abbott Cat #: 30-804931) or DAPI III (Abbott Cat#: 30-804932)

### Solutions

* 1. 20X SSC solution: Mix thoroughly 132 g 20X SSC in 400 ml purified H2O. Add purified H2O to bring final volume to 500 ml. Measure pH and adjust to pH 5.3 with HCl. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.
  2. 2X SSC solution: Mix thoroughly 100 ml 20X SSC (pH 5.3) with 850 ml purified H2O. Add purified H2O to bring final volume to 1 liter. Measure pH and adjust to pH 7.0±0.2 with NaOH. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.
  3. 2X SSC / 0.1% NP-40 wash solution: Mix thoroughly 100 ml 20X SSC (pH 5.3) with 850 ml purified H2O. Add 1 ml NP-40. Add purified H2O to bring final volume to 1 liter. Measure pH and adjust to pH 7.0±0.2 with NaOH. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.
  4. Ethanol Solutions (70%, 80%, 95% and 100%): Prepare v/v dilutions of 100% ethanol with purified H2O. Between uses, store covered at ambient temperature. Discard stock solutions after 6 months.
  5. 0.4X SSC / 0.3% NP-40 wash solution: Mix thoroughly 20 ml 20X SSC (pH 5.3) with 950 ml purified H2O. Add 3 ml of NP-40. Mix thoroughly until NP-40 is completely dissolved. Add purified H2O to bring final volume of the solution to 1 liter. Measure pH and adjust pH to 7.0-7.5 with NaOH. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

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### Procedure

### 1. Specimen Requirements

Neoplasia sample volumes are dependent on the white blood cell count of the sample. Generally, about 1ml of bone marrow or neoplastic blood is sufficient for FISH. Routine FISH tests are performed on the unstimulated A culture, when possible. FISH is performed on the sorted cells (when possible) for plasma cell disorders.

FISH can also be performed on Peripheral Blood, Solid Tissue Samples, Amniotic fluid and Paraffin Embedded Tissue, as long as there is an adequate amount of cells present.

**2. Probe Preparation**

1. Prior to use, thaw reagents at ambient temperature, vortex, and centrifuge each tube 2-3 seconds using a standard bench-top microcentrifuge.
2. Probes are generally premixed and ready for use either by manufacturer or FISH specialist. The FISH specialist will premix the Abbott probes that require it. All probes with new lot #’s will be validated prior to use.
3. For a 10ul vial of probe, mix 70ul of buffer with 20ul of H20. When mixed a 200ul tube should be used and labeled with contents, lot number, date expired and date prepared. Alternatively, probes can be made for use at the time needed at the ratio of: 7 µl of Hybridization Buffer, 1 µl LSI DNA probe, and 2 µl purified H2O at room temperature.
4. Centrifuge for 1-3 seconds, mix and then re-centrifuge before each use.

**3. Preparing the Sample Target**

* + - 1. Prepare slides from fixed cell pellet (blood, bone marrow, amniotic fluid, tissues)
      2. Metaphase or interphase nuclei preparation should be well spread and relatively free of cytoplasm
      3. Mark hybridization areas with a diamond tipped scribe on the specimen slide, if necessary
      4. Place 2xSSC jar in a 73±1°C waterbath approximately 60 min prior to use to bring the solution to temperature. *Note: add the coplin jar to a cooled water bath and then turn water bath on to avoid cracking the coplin jar*
      5. Put **up to 4 slides** in 2xSSC for 1 minute

f. Dehydrate in a standard ethanol series (1 min each in 70%, 80%, 95%, & 100%)

**4. Hybridization** (use rapid hybridization with ThermoBrite automated chamber)

1. Power on the ThermoBrite and soak pads located under the lid with H2O from squeeze bottle if leaving the slides on the ThermoBrite for more than 20 minutes.
2. Following dehydration step above, remove slides from the 100% EtOH. Wipe the back of each slide with a Kimwipe and blot the end on a paper towel.
3. Allow EtOH to evaporate from the surface of the slides or air dry, then apply 10 µl of prepared probe to each 22 X 22 mm target, followed by a clean coverslip.
4. Run-out any large air bubbles trapped under the coverslip with forceps.
5. Using a syringe, apply paper cement to seal all edges of the coverslip.
6. Run ThermoBrite program according to manufacturers’ specifications. Once the temperature has come back to 37C on the ThermoBrite, transfer slide from ThermoBrite to humid box in 37°C incubator for hybridization.

*Note: A 16-hr hybridization is optimal; however, some probes may be ready in as little as 4 hr; smaller LSI probes need at least 6-8 hr of hybridization time in STAT or urgent situations.*

**5. Slide washing**

1. 4-24hr later, prepare Coplin jar with **wash I** (0.4X SSC/0.3% NP-40) (discard after 1-4 days of use) and bring to 73+/-1°C in waterbath.
2. Prepare Coplin jar with **wash II** (2X SSC/0.1% NP-40) at room temperature. Discard after 1 week.
3. Remove paper cement seal and the coverslip and immediately place into wash I (0.4X SSC/0.3% NP-40), for 2 minutes, agitating the slide for several seconds - **maximum four slides at one time**.
4. Place slide in 2X SSC/0.1% NP-40 (Wash II) at room temperature for approximately 1 minute, agitating for 1-3 seconds.
5. Blow dry slide, keep slide in the dark, place in a slide folder.
6. Dry slide completely; apply 10 µl of DAPI II or DAPI III (Vysis/Abbott) counterstain to each target area with a pipetter, followed by a fresh coverslip.

g. Run-out any large air bubbles using forceps and blot the excess counterstain from the slide with a paper towel. The slides can be read immediately or stored in the refrigerator.

**6.** **Scoring**

1. Using the DAPI filter and the 10x objective, choose an area of the slide containing a large concentration of cells, for ease of scoring, make sure cells are not overlapping.
2. Change to the 100x objective and begin scoring using the appropriate filter set.
3. Two readers score 100 consecutive scorable cells each, skipping overlapped cells and cells without a clear signal pattern.
4. Keep track of signal patterns using digital cell counter and record results on the FISH score sheet.
5. Counts should be extended if clinically indicated or if requested by physician

***Note:***  *For complete FISH scoring guidelines see Cytogenetics Testing Work-up Guidelines Procedure: 400-06-01-01*

REFERENCES

* 1. American College of Medical Genetics Laboratory. Standards and Guidelines for Clinical Genetics Laboratories, 2nd ed. Bethesda, MD: ACMG, 1999.
  2. Vysis LSI protocol, revision 6/10

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Cytogenetics Supervisor

Cytogenetics **-** UWMC

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: Fluorescence in Situ Hyridizaton (FISH) – Detection of Numerical, Microdeletions, Translocations or Rearrangements, and Amplification abnormalties

Procedure / Policy Number: 400-04-01-04

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