**UW Medicine - Pathology**

400-04-01-02

Interphase Fluorescence in Situ Hybridization (FISH) on Uncultured Amniocytes Procedure

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| Adopted Date: 09/1994Review Date: 06/12/09, 05/16/11Revision Date: 08/27/12, 9/17/13 |

PURPOSE

This procedure confirms or rules out aneuploidy for chromosomes 13, 18, 21, X, and Y. Results can be obtained within 24 hours, providing rapid, preliminary information. When possible, results must be confirmed with standard cytogenetics.

PROCEDURE

### Materials and Equipment

### Standard bench top laboratory centrifuge

### 37°C incubator

### 15 ml clear, plastic Falcon centrifuge tubes (Falcon #352095)

### Clean glass microscope slides

### Vysis AneuVysion kit (Cat# 161075)

### 22 x 22 mm coverslips

### Rubber cement

### Micropipetor and tips

### Diamond pen

### Coplin jars

### Fluorescence microscope, equipped with recommended filters

### Reagents and Solutions

1. 2X Trypsin/EDTA solution (37°C)

Mix 10 ml of 10X stock trypsin-EDTA (Invitrogen #15400054) + 40 ml sterile PBS (Gibco #14190-136)

1. Carnoy's fixative solution (3:1 methanol:acetic acid), make fresh, keep at room temperature.
2. 2X SSC
3. 0.4X SSC + 0.3% NP-40
4. 2X SSC + 0.1% NP-40
5. 70% ethanol
6. 95% ethanol
7. Absolute ethanol
8. DAPI II (comes in Vysis AneuVysion kit with probes)
9. Solutions instructions:
10. 20X SSC (for 500 ml of 20X SSC, pH 5.3)
	1. Add 132 g 20X SSC to 400 ml H2O and mix thoroughly.
	2. Adjust pH at room temperature with a pH meter to 5.3 using concentrated HCl and adjust to final volume of 500 ml.
	3. Filter through a 0.45 micron pore filtration unit.
	4. Store up to six mo at room temperature.
		1. 2X SSC/0.1% NP-40 (for 1 L)
			1. Add 100 ml 20X SSC (pH 5.3) to 850 ml purified H2O.
			2. Add 1.0 ml NP-40.
			3. Adjust pH to 7.0-7.5 with NaOH using a pH meter.
			4. Add H2O to bring final volume of the solution to 1 L.
			5. Store up to 6 mo at room temperature.
		2. 0.4X SSC/0.3% NP-40 Wash Solution
			1. Mix thoroughly 20 ml of 20X SSC with 950 ml purified H2O.
			2. Add 3 ml NP-40. Mix thoroughly until NP-40 is dissolved.
			3. Adjust pH to 7.0 - 7.5 with NaOH.
			4. Add purified H2O to bring final volume to 1 L.
			5. Store at ambient temperature.
			6. Discard stock solution after 6 mo, or if solution appears cloudy or contaminated.
			7. Store up to 6 mo at room temperature.
		3. Ethanol Wash Solutions
			1. For final concentrations of 70%, 95% and 100%.
			2. Prepare v/v dilutions of 100% ethanol with H2O.
			3. Use dilution for up to seven days and then discard.
			4. If solution evaporates or becomes diluted, replace with fresh solution.
			5. Between periods of use, store at room temperature.

### Procedure

***Note***: No mouth pipetting. Adhere to sterile techniques. Use tissue culture hood [Biosafety Hood (BioGard)]. Use gloves.

1. Centrifuge whole amniotic fluid (5 ml) in a 15 ml plastic centrifuge tube, for 10 min at 1000 RPM. Note size and color of pellet. Greenish, brownish, and bloody-appearing samples may contain contamination from maternal cells, which may affect FISH results. FISH is not recommended for bloody samples for this reason. If specimen is received late in the day, 5 ml aliquot can be kept refrigerated over night.
2. Pipette off supernatant and resuspend the pellet by mixing well. Add 3 ml of room temperature 2X Trypsin/EDTA and mix the sample thoroughly. Incubate at 37°C for 20 min.
3. Slowly add 2ml of Carnoy's fixative and mix gently.
4. Centrifuge for 10 min at 1000 RPM. Remove supernatant, gently resuspend pellet and slowly add 2-4ml of fixative. After a minimum of 10 min, centrifuge for 10 minutes and move to slide making.

### Hybridization

* + 1. Centrifuge tube for 10 min, pipette off fixative and add enough fresh fixative to make at least one slide with 2 drops. If possible, make another slide for back up. Etch, using a diamond pen, on either side of the 2 drops on slide. Age slides at 90°C for 20 minute followed by 1 minute in 2X SSC warmed to 73°C. Then dehydrate in ethanol series.
		2. Vysis AneuVysion probes are used. Remove from freezer and allow to warm for 5 minutes in a covered box. Apply 8 µl of LSI 13/21 probe to one target area and 8 µl CEP 18/X/Y to the other target area. Cover each target area with a 22 x 22 coverslip and seal with rubber cement. Denature for 2 min at 73°C on HYBrite thermal controller. Incubate overnight in a moist chamber at 37°C.
		3. After overnight incubation, turn on water bath and place Coplin jar with 0.4X SSC + 0.3% NP-40 and heat to 73°C. Remove coverslip and wash slide for 2 min. Place in 2X SSC with 0.1% NP-40 and rinse briefly. Air dry and apply 8 µl of DAPI II, coverslip. View under fluorescence microscope.

### Results, Guidelines and Controls

1. Score 50 nuclei for each probe. At least two technologists should score unique cells for each case whenever possible.
2. Choose intact nuclei that are not overlapping or pushed against another.
3. Capture color images of a nucleus for each probe mix: 1 hard copy if normal, 2 if abnormal.
4. A minimum of 90% consistency of finding is needed for diagnosis. If consistency is less than 90%, the hybridization must be repeated with control slides. This is indicated on the FISH report. **See IFISH Scoring Guidelines procedure and Vysis protocol sheet for scoring guidelines.**
5. Use proper ISCN nomenclature for ish diagnostics as outlined in the ISCN (2009).

REFERENCES

1. Roche Laboratories: [http://www.roche-applied-science.com/fst/publications.htm?/PROD\_INF/MANUALS/InSitu/InSi\_toc.htm]
2. Trask, B., Pinkel, D., Gray, J.W. et. al. Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. Cold Spring Harbor Symp. Quant. Biol. 51(Pt 1):151-157, 1986.
3. Birren, B., Green, E., Heiter, P., Myers, R. et al.: editors Genome Analysis: A Laboratory Manual Chapter 7, Fluorescence in situ Hybridization, by Barbara Trask. Cold Springs Harbor Press, 1999.
4. ISCN (2005): *An International System for Human Cytogenetic Nomenclature*: Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature, eds., Shaffer, L.G., Tommerup, N., S. Karger, Basel, Switzerland, 2005.

Written By: Director Approval:

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 Cytogenetics Supervisor

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Procedure / Policy Number: 400-04-01-02

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\_\_\_\_\_\_\_\_\_\_\_, 20\_\_\_\_.

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Medical Director Medical Director Signature or Date Reviewed

Print or Designee Designee

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