**UW Medicine - Pathology**

400-01-01-03

Specimen Testing Protocols

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| Adopted Date: 08/2003  Review Date: 09/2005  Revision Date: 08/2012 |

PURPOSE

To ensure proper specimen collection.

PROCEDURE

### Amniotic Fluid Samples

### Amniocentesis is generally performed between 15-18 weeks gestation but can be performed as early as 10 weeks. Ideally, 15-30 ml of fluid should be collected under sterile conditions using sterile containers approved for cell culture. Every effort should be made to salvage less than optimal samples. Samples should be transported at room temperature. Physicians should be advised to discard the first 1ml of fluid as it is most likely contaminated with maternal cells.

### Chorionic Villi Samples

1. Generally, a minimum of 10mg of sample is needed to obtain direct and long term cultured cell results. 20mg or more is ideal. Specimens should be placed in sterile transport media, provided by the Cytogenetics Laboratory, and kept at room temperature.

### Peripheral Blood Samples

1. **Cytogenetics** analysis (karyotype or FISH):
   1. **Adults**: 5 to 10 ml of peripheral blood collected in a sterile syringe or vacutainer containing preservative-free sodium heparin (green top, blue top and sometimes red top tubes).
   2. **Infants:** Draw 1-3 ml venous blood into a heparinized syringe (10-20 USP units preservative-free sodium heparin per ml of blood) or a small green-top vacutainer tube with heparin.
      1. **Molecular** analysis:
         1. **Combined Fragile X DNA test and routine karyotype**: 5 ml of peripheral blood in a Sodium Heparin vacutainer for cytogenetics and 5 ml in EDTA vacutainer tube for fragile X DNA.
         2. **Male infertility Y PCR**: 5-8 ml peripheral blood in EDTA purple-top vacutainer.
      2. **SCE or Breakage Study**: 5 to 10 ml of peripheral blood collected in a sterile syringe or vacutainer containing preservative-free sodium heparin. *A normal control sample (same age and sex as patient is preferred).*

### Neoplasia specimens

1. **Bone Marrow**

1-3 ml of bone marrow collected sterile in a sodium heparin vacutainer or sterile syringe containing sodium heparin. Keep sample at room temperature

1. **Leukemic blood**

5 to 10 ml of leukemic blood collected in a sterile syringe or vacutainer containing preservative-free sodium heparin (green top, blue top and sometimes red top tubes). Keep sample at room temperature

*Note: Leukemic blood can be used in place of bone marrow when blasts are present in the blood*.

1. **Bone Core Biopsy**

Specimen should be immediately placed in a sterile vial with 3 to 5 ml **Tissue Transport Medium** (\*see below). Keep sample at room temperature

### Urine

1. Obtain 40-100 ml voided urine in a sterile jar or cup. Transport specimen at room temp or on ice. Refrigerate and add 70% ethanol 1:1 (v:v) or Carbowax preservative 2:1 (v:v), if not shipped immediately.

### Solid Tissues

1. **Skin Biopsy**. 2-4 mm2 sample of skin (approximately 2 mm x 2 mm) in sterile vial(s) containing **Tissue Transport Medium** (\*see below). Use separate vials, if separate sites of biopsy, and label accordingly. **DO NOT PUT** **specimen in formaldehyde, alcohol or saline.** Send on ice or refrigerate if not shipped immediately.
2. **Solid Tumors, Abortus or Autopsy Material**  (spontaneous abortion, fetus, stillborn, placenta, etc.): Samples should be obtained in sterile tubes or vials containing transport media. Physicians should be advised to collect in a sterile environment when possible. Samples from different sites (placenta, skin) should be transported in separate vials and labeled accordingly. **NOTE: DO NOT PUT specimen in formaldehyde, alcohol or saline. DO NOT SHIP ENTIRE FETUS.** Send on ice or refrigerate if not shipped immediately.

\***Tissue Transport Medium**: Cytogenetics will supply transport medium if called in advance. **Lactated Ringer's solution or viral transport medium** are acceptable alternatives if shipping time will not exceed 24 hr. **DO NOT USE** saline, 5% dextrose, or tissue culture medium buffered with bicarbonate.

### Specimen Volume Requirements

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| **SPECIMEN TYPE** | **Ideal amount of specimen** | **Minimum amount required** |
| **AF (amniotic fluid cytogenetics)** | Routine: 20 ml  IFISH: 5 ml | 5 ml (if no IFISH, AFP, or ACHE) |
| **Transit time:** less than 7 days.  To remedy insufficiencies: Call sender 1) Attempt second tap; 2) Expect delayed results and fewer cultures | | |
| **CV (chorionic villi cytogenetics)** | Routine: 20 mg villi transport medium | 5 mg (useable villi after cleaned) |
| **Transit time:** less than 5 days.  To remedy insufficiencies: Few or no villi: Inform MICC, PDC genetic counselor, or referring physician.  If no direct prep, do IFISH and notify counselor or physican | | |
| **PB (peripheral blood cytogenetics)** | Adults: 5 to 10 ml sodium heparin  Infants: 1 to 2 ml sodium heparin | Fetus: .6-.8 if fetus |
| **Transit time:** less than 7 days.  To remedy insufficiencies: If newborn, 1) try IFISH for aneuploidy. If adult, 2) redraw if needed; 3) FISH per Director's/Supervisor's decision | | |
| **NE (neoplastic bone marrow cytogenetics)** | 1 ml bone marrow or 5 ml blood (w/ 5% blasts) | Varies depending on cell count (minimum of 5 million cells total) |
| **Transit time:** less than 5 days.  To remedy insufficiencies: Call sender | | |
| **TR (tumor cytogenetics)** | 0.5 to 1.0 cm2 in transport medium |  |
| **Transit time:** less than 7 days.  To remedy insufficiencies: Attempt to determine why specimen didn't grow. | | |
| **ST (solid tissue, skin cytogenetics)** | 2+ cm2 in transport medium | 0.5 to 1.0 cm2 in transport medium |
| **Transit time:** less than 7 days refrigerated and in transport media. Less than 2 days if not correctly stored.  To remedy insufficiencies: 1) Set up only one culture with 2 flasks; 2) One culture with 1 flask; 3) Delayed results/culture failure are possible. IF FROZEN, set up as usual, FAILURE likely. If exposed to formalin, FAILURE likely. | | |
| **UroVysion (bladder cancer monitoring -IFISH)** | 40-100 ml urine or bladder washing | 20 ml |
| **Transit time:** less than 7 days if refrigerated.  To remedy insufficiencies: Re-obtain specimen. | | |
| **Y-PCR** | 3 to 5 ml EDTA whole blood |  |
| **IFISH/FISH** | Fixed Specimen |  |
| **Transit time:** less than 10 days.  To remedy insufficiencies: 1) Verify hybridization conditions on control slides; 2) prepare more slides. | | |
| **Breakage Study/X-Inactivation**  (Fanconi anemia) | 1-2 ml infant; 2-5 ml adult  Need Control: age and sex matched |  |
| **Transit time:** less than 7 days.  To remedy insufficiencies: 1) Verify dilutions of reagents; 2) re-obtain specimen. | | |

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Cytogenetics Supervisor