**UW Medicine - Pathology**

400-02-01-06

Solid Tumor Cultures

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| Adopted Date: 09/04/91  Review Date: 06/12/09, 5/11/11  Revision Date: 01/28/2013, 9/30/13 |

PURPOSE

Aid in diagnosis/prognosis of sarcomas by identifying chromosome abnormalities specific to a type of tumor, e.g., as in Ewing's, synovial, rhabdomyosarcomas, chondrosarcoma, lipoma, and liposarcoma.

PROCEDURE

### Specimen Requirements

* + - 1. Tumor should be collected sterile
      2. Place specimen in a sterile centrifuge tube or other sterile container containing transport media and keep refrigerated. Deliver the specimen to the laboratory ASAP.

### Materials and Equipment

* + - 1. Laminar flow hood [Biosafety Hood (BioGard)]
      2. CO2 incubator 37oC.
      3. Water bath
      4. Inverted microscope
      5. Centrifuge
      6. Sterile forceps.
      7. Scalpel blades.
      8. Sterile plastic centrifuge tubes (Falcon #2095).
      9. Sterile 25-mm2 tissue culture flasks.
      10. 5-ml and 10-ml sterile graduated pipettes.
      11. 1.5-ml Eppendorf tube specifically labeled for freezing.
      12. Slide flask (NUNC Cat. #170920).

### Reagents and Solutions

* + - 1. Alpha MEM Earle's salts nucleosides (Irvine Scientific, Cat. #9144).
      2. Amniomax C-100 (Gibco Cat. #17002-017).
      3. Amniomax supplement (Gibco Cat. #17002-017).
      4. RPMI 1640 (Sigma Cat. #R8758).
      5. PHA (Burrows-Wellcome Cat. #HA-15).
      6. Fetal bovine serum.
      7. Penicillin-streptomycin-glutamine (Gibco Cat. #10378-032).
      8. Collagenase (Worthington type 2)
      9. Fungizone (Gibco Cat. #15295-017). Concentration 2.5 µg/ml.
      10. Gentamycin (Gibco Cat. #15710-023). Concentration 0.2 ng/ml, or Gentamycin sulfate (Lymphomed Cat #400972 G/A-92).
      11. Hank's balanced salt solution (HBSS) (Irvine Scientific Cat. #9228).
      12. Colcemid (Gibco Cat. #120-5210AD).
      13. Carnoy's fixative (3 parts absolute methanol:1 part Glacial acetic acid.
      14. Versene 1:5000 (Gibco Cat. #15040-066).
      15. Trypsin stock (20 ml trypsin-EDTA 10X (Gibco Cat. #6105405) rehydrated with 20 ml autoclaved distilled H2O working solution 10 ml stock + 90 ml PBS.
      16. Trypan blue (Gibco Cat. #15250-061).
      17. 1 M HEPES (Gibco Cat #
      18. Reagents and Media:
          1. 100 ml alpha MEM (with Earl's salts and nucleosides): Prepare two different lots at a time, labeled A and B to use in different vessels for each case.

20 m1 fetal bovine serum

100 ml Amniomax C-100

Amniomax supplement

1 ml penicillin-streptomycin-glutamine

0.4 ml Normocin (Cat # ant-nr-0, Invivogen) (Stock= 50 mg/ml Liquid)

One small aliquot of each bottle must be incubated at 37°C for at least 24 hr before using the media to make sure the media is not contaminated. Whenever possible, overlap new media lots between different cultures in a case to verify that a new lot can support growth.

Record media lot in case log book.

Media shelf life is about two weeks when stored at 4°C.

* + - * 1. Transport medium:

100 m1 alpha MEM

10 m1 fetal bovine serum

2.7 ml of 1 M HEPES buffer

2 ml penicillin-streptomycin-glutamine

0.2 ml Normocin

0.4 ml Fungin

Aliquot 5-7 ml into 15 ml Falcon tubes. Label with 1 x 2" labels. Include expiration date.

Shelf life is 3 mo at 4°C.

* + - * 1. Tissue wash:

100 ml PBS (or Hank's)

0.2 ml penicillin-streptomycin

0.2 ml Normocin

0.4 ml Fungin (10 mg/ml) (Cat # ant-fn Invivogen)

Shelf life is 1 mo at room temperature; 2 mo at 4°C

* + - * 1. Collagenase pretreatment:

Reconstitute 1 vial (100mg) collagenase in 100 ml Alpha MEM bottle. Mix well.

Filter using a Nalgene filter chamber (150ml, 0.2µm).

Aliquot 4ml into 15ml blue top tubes. Label with a 6 month expiration and put in freezer door rack. (Lab tech usually has these prepared)

### Culture

* + - 1. **Log in**: When a sample is received it is logged in the tumor log-book and a case number is assigned: TR/last 2 digits of a year/consecutive number, 4-digit accession number, e.g., **TR04-0052**. Log on the board, GCS and on worksheet. If tumor comes after hours, put it in the refrigerator until the next morning and leave a note on the white board for the wet lab team member. If an indication for a specimen is not among the usual indications for this type of specimen or if an indication is ambiguous or unclear, the technologist must call or have front office staff call the sending physician to clarify before setup. Every effort should be made to avoid mislabeling of samples including ensuring that only one specimen is set up at a time (**ONE SAMPLE IN THE BIOSAFETY HOOD (BioGard) AT A TIME**).
      2. **Culture set up**
         1. Place tumor and transport media in a Petri dish using scalpel and forceps. Dissect any different-appearing portions. Record tissue appearance (e.g., red, soft, etc.). All centrifuge tubes, Petri dishes, flasks should be labeled with case number (accession number), date and culture number (e.g., TR/last 2 digits of year/consecutive number and culture number A, B, C, etc.), and the patient’s first initial and first three letters of last name (e.g., Mary Jones = **MJON**)

* 1. If microarray is requested as reflex or first order, a portion of solid tissue is split with the array lab. 50-150mg of tissue is needed. If there is inadequate sample to split, two confluent T25 flasks or three 70% confluent T25 flasks are grown up for the array studies.
     + 1. Rinse the portions of tumor with tissue wash.
       2. Keep transport media in a centrifuge tube for presence of any remaining cells that have separated out. Spin down and re-suspend in growth medium.
       3. Place each tissue in a Petri dish (pieces should be cut into small portions approx 5-7 mm2 (the size of a small pea). Set up at least 3 coverslips and 1 backup flask from each morphologically different tissue type. The number of coverslips and flasks depends on the size of the sample, but if it is very small, set it up in 1XT-12.5 flask.
       4. Complete the centrifuge tube with collagenase (0.5 ml) with 4.5 of complete medium and take 1-2 m1 of this mixture and place in the Petri dishes with the tumor (to keep moist).
       5. Mince the tissue finely with 2 scalpels.
       6. Add 1 ml of medium to the minced tumor and transfer everything to the centrifuge tube, and add approximately 5 ml to the cutting dish for growth.
       7. Place the centrifuge tube in a 5% CO2 for 2 hr as back-up at 37°C.
       8. When enzyme treatment is complete tissues will appear loose. Pipette the mixture, add 5 ml of media and centrifuge cells out at 1000 rpm for 10 min.
       9. Remove supernatant, leaving 1 ml; re-suspend each tissue and place on 3 coverslips and 1 flask (T25 flask ), add 4 ml of media (media A and B) . Incubate at 37°C in 5% CO2. If there is not enough specimen, only set up one T-12.5 flask.
       10. Each flask should have a label generated from GCS containing Patient Name, date, culture letter.
       11. Check after 1-2 days flood coverslips and check for growth and possible harvest.
       12. Feed the flasks twice weekly by pouring off media; feed with 5 ml of media.

### Pathology Report

* + - 1. Prior to signout, a pathology report should be obtained via Mindscape, Orca database, or powerpath (patient name and surgical pathology number required) and provide printout and file to sign-out faculty to review.
      2. If unusual pathology or if not a sarcoma, sign-out faculty should discuss case with staff and/or pathologist in order to stop unnecessary work-up.

### Harvest

* + - 1. Reagents and solutions
         1. Colcemid (Gibco) 10 µg/ml
         2. Hank's 1X saline
         3. Fresh trypsin-EDTA (2 ml of l0X Gibco trypsin-EDTA diluted in 18 ml Hank's 1X saline) for cultured harvests only.
         4. Hypotonic solution.: 1.2 g KCl (potassium chloride): 0.4g Na3C6 sodium citrate dehydrate); 400 ml of sterile H2O. good for 1month at room temperature
         5. Fixative: 3:1 methanol and acetic acid, freshly made before use.
      2. Harvest
         1. When the flask shows growth (more than 50% of the surface with a good mitotic index). Add 60ul-100ul colcemid to flask for 3-5 hr.
         2. Pour medium into a centrifuge tube (label the tube with case number and date).
         3. Add 2 ml trypsin-EDTA to flask and incubate for 5 min at 37°C. Monitor cell detachment microscopically. Tap flask on bench top to help cell detachment.
         4. When cells are detached, pipette with a 2 ml pipette to separate cells and add suspension to tube; re-feed the flask, gas and incubate for future growth.
         5. Centrifuge tubes at 1000 rpm for 10 min. Remove supernatant. Re-suspend gently with Pasteur pipette.
         6. Add 1 ml warm 0.4% KCl/NaCitrate and pipette (very gently) to mix. Add 4-5 ml 0.4% KCl/NaCitrate and place the centrifuge tube at 37°C in incubator for 20 min.
         7. Add 4-5 drops fresh 3:1 fixative; pipette to mix and centrifuge as before.
         8. Remove supernatant. Re-suspend gently and add 1 ml of fixative slowly; re-suspend and complete with 4-5 ml fixative.
         9. Let stand in fixative at least 30 min in the refrigerator or until ready for slides. (Cells look better after overnight refrigeration or freezing before slides are made.)
         10. Slides are made as usual, bake at 90-95°C for 45 min and G-band with trypsin.
         11. A second harvest from a different culture should be performed even if enough material was obtained from the first one.
      3. Direct harvest- Direct harvests have a very low success rate and are not done routinely.

***Note****:* Some tumors are actively dividing when they arrive, others never attach to the flask and grow in suspension, and most shed into the transport media. In these cases a direct, or overnight, harvest should be performed. Good examples of these situations are:

* All small round blue cell tumors, especially neuroblastomas.
* Any tumor with a high mitotic index on the pathology report.
* Tissue suspected for Ewings should have a direct harvest
* Suspected lymph node or floating cells which are not adhering to the flask.

***Note:*** If the specimen arrives on a Friday or weekend, and there is inadequate amount of time to perform the harvest below. The specimen may be processed the next day with the Neoplasia A Culture harvest procedure (400-02-01-04).

* 1. Add 0.01 ml of colcemid to the media.
  2. Place the centrifuge tube into the incubator for 3-4 hr or overnight.
  3. Centrifuge tube for 10 min at 1000 rpm.
  4. Remove supernatant to 1/4 ml pipette to re-suspend the pellet.
  5. Add 1-5 ml of warm hypotonic (0.4% sodium citrate with potassium chloride).
  6. Let sit in the incubator for 15-20 min.
  7. Add 4-5 drops fixative. Centrifuge.
  8. Remove supernatant to 1/4 or 1/2 ml. Pipette to re-suspend pellet again. Add 4-5 drops fixative, mix, then add up to 3-5 ml fixative.
  9. Place in freezer from 20 min to overnight. Centrifuge cells, remove supernatant and make slides using fresh fixative.

### Solid Tumor Chromosome Analysis

Usually at least 2 different cultures are harvested and analyzed (if a suspension 24-hr culture is available, both attached and suspension cultures should be analyzed). If only one culture is available, all 20 cells may be from a single flask. A minimum of three cells are karyotyped. If multiple clones are present, two karyotypes are needed per clone.

***Note****:* No mouth pipetting. Adhere to sterile techniques. Use tissue culture hood [Biosafety Hood (BioGard)]. Use gloves. Use multiple incubators when possible. All glassware in contact with blood must be autoclaved after use. Bleach, fix or autoclave all labware in contact with specimen.

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Written By: Director Approval:

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Cytogenetics Supervisor

**UW Medicine - Pathology**

**Cytogenetics - UWMC**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: Solid Tumor Cultures

Procedure / Policy Number: 400-02-01-06

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