**UW Medicine - Pathology**

400-02-01-12

Fetal Bovine Serum Testing Protocol

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| Adopted Date: 05/15/05Review Date: 06/08/10Revision Date: 04/13/11 |

PURPOSE

To ensure Fetal Bovine Serum (FBS) has the ability to support cell growth.

PROCEDURE

### Specimen Requirements

Select a nearly confluent T25 flask from a recent amniocyte culture or solid tissue culture. Two different cultures should be chosen and tests run side by side.

***Note:*** Patient identifying information must be removed from flasks.

### Materials and Equipment

* + - 1. Inverted phase contrast scope
			2. 5-ml sterile serological pipettes
			3. Sterile glass pasteur pipettes
			4. Hemocytometer
			5. Sterile T75 flasks
			6. Standard centrifuge
			7. Light microscope with 10X and 40X objectives
			8. Timer

### Reagents and Solutions

1. Hank’s Balanced Salt Solution (HBSS) without calcium and magnesium (Life Technologies Inc. Gibco #14170-062). Store at 2°C to 8°C after opening.
2. Trypsin stock: 20 ml trypsin ‑ EDTA 10X (Gibco Cat. #610-5405) rehydrated with 20 ml autoclaved distilled H2O working solution 10 ml stock + 90 ml PBS.
3. Alpha MEM Earle’s salts nucleosides (Irvine Scientific cat. #9144)
4. Amniomax C-100 (Gibco)
5. Penicillin-streptomycin-glutamine (Gibco Cat. #10378-016)
6. Normocin (Invivogen Cat# ant-nr-0)
7. Solid Tissue Medium:

***Note:*** Prepare two different lots at a time, labeled A and B to use in different vessels for each case.

* 1. 100 ml alpha MEM (with Earle's salts and nucleosides) (Irvine Scientific Cat. #9144)
	2. 20 ml fetal calf serum
	3. 100 ml Amniomax C-100 (Gibco Cat. #17001-017) + Amniomax supplement (Cat. #17002-105)
	4. 1ml Pen/Strep + L-glutamine (Gibco BRL Cat# 10378-016) (stock = 10,000 U/ml pen; 10,000 µg/ml strep; 20.2 g/ml glutamine)
	5. 0.4 ml Normocin

***Note:*** 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 2 weeks when stored at 4°C.

### Procedure

1. Prepare tissue culture media as usual for solid tissue specimens leaving out the FBS. Split the A and B media between two flasks and add 10 ml of the FBS lot that you want to test. Label the media flasks with FBS lot number.
2. Select a nearly confluent T25 flask from a recent solid tissue culture.
3. Working in the tissue culture hood [Biosafety Hood (BioGard)] and using sterile technique, remove and discard medium from flask.
4. Wash flask with 5 ml of Hank’s balanced salt solution (HBSS) and remove. Repeat one more time pouring the second wash into a labeled 15-ml falcon tube.
5. Add 1.0 ml of trypsin solution to the flask, swirl briefly and let sit for 1-2 minutes.
6. Knock flask sharply against counter repeatedly to release cells.
7. Pour the appropriate tube of HBSS from the 15-ml falcon tube into the flask, swirl the entire solution around in the flask, then pour all of the contents back into the labeled 15-ml tube.
8. Centrifuge the 15-ml tube for 10 min at 1000 rpm.
9. Remove as much of the supernatant as possible without disturbing the cell pellet. Re-suspend cells, then add 5ml of HBSS and mix thoroughly. Cells should remain in HBSS for a minimal time.
10. Use a sterile glass Pasteur pipette to place a drop of cell suspension into the counting chamber of the hemocytometer.
11. Count cells under the 40X objective. Calculate the amount of cell suspension containing 500,000 cells (see calculations).
12. Label two T25 flasks for each brand/lot of serum to be tested. Inoculate each flask with 500,000 cells.
13. Add 5 ml of appropriate media with the lot of FBS you are testing. Place in 37°C incubator for 2 to 3 days.
14. After 2 to 3 days, determine the number of cells in each flask by following steps 1 through 11 above. Expose each flask to trypsin for exactly the same amount of time.

***Note:*** DO NOT allow flasks to reach confluency as this will bias the calculations.

1. Repeat the entire procedure with 2 to 3 different solid tissue cultures. These can be done at the same time.

***Note:*** FBS lots that perform poorly in the first two rounds may be eliminated from testing in later rounds.

1. Compare the growth rates of the different brands and lots of FBS and select the best for purchase.

### Calculations

* + 1. Count the cells in each of four large corner squares of the hemocytometer and total them.
		2. Convert the number of cells counted to number of cells per ml of sample:

(total # cells counted)(50\*)1000 = # cells/ml of sample

\*hemocytometer conversion constant

* + 1. Calculate quantity of sample containing 500,000 cells by solving for X:

 #cells (X ml of sample) = 500,000 or

ml of sample

X ml of sample = (500,000 cells) ml of sample

 #cells

Record results on form FBS testing Calculation sheet, order correct lot of FBS and file in the media validation notebook. (Quality Assurance Notebook).

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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 Cytogenetics Supervisor