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# Supplemental Materials

# for

# Introducing Mammalian Cell Colony Formation in the Undergraduate Biology Laboratory

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## Appendix 1: Safety Considerations and Precautions

Work with mammalian cells in culture such as HeLa cells requires the use of a Biosafety Level 2 (BSL-2) cell culture lab that is equipped with specialized equipment such as biosafety cabinets. These are often shared-use facilities available to students and instructors. All individuals using this facility should be properly trained in the safe use of materials and facilities.

The BSL-2 experiments described in this article were piloted and developed in an upper level Cancer Biology course. Before working in this BSL-2 setting, all students had previously conducted experiments and demonstrated proficiency with BSL-1 measures in the context of pre-requisite foundation courses. In these required courses, students used common and innocuous bacterial and yeast strains such as *E. coli* and *S. cerevisiae*. Student's ability to safely work in BSL-1 settings was assessed by multiple laboratory practical tests. To prepare students to work in a BSL-2 setting in the context of upper level courses, faculty taught and demonstrated necessary lab skills and aseptic techniques. Student mastery of BSL-2 techniques was assessed using practical tests. Students had to demonstrate mastery of these practices before performing colony formation assays.

Safety Training Requirements for Students and Instructors:

**1) Online CITI training.** (https://www.citiprogram.org/index.cfm?pageID=154&icat=0&ac=0)

When registering for an account, select your institution as the *Organization Affiliation*.

When asked if you would like Continuing Education Credits, choose no.

Select the curriculum under "Human Subjects Research" called

“Biomedical Research Investigators” under Question 1.

Select “Biosafety Complete Training” under Question 5.

Select “Not at this time” or “No” for the other Questions.

Complete the following modules in the Biosafety Complete Training series.

- Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogen Standard,
- Hepatitis B Virus (HBV) Vaccination,
- Labels and Engineering Controls,
- Universal Precautions and Work Practices

Once you complete these modules, send your completion certificate to your instructor.

**2) Hepatitis B Vaccine or Declination Form**

Vaccination against the Hepatitis B virus (HBV) is provided free of charge to students and instructors who have exposure to human blood, blood products, certain human body fluids, tissues, organs, and primary cell lines. The HBV vaccination is a series of 3 shots given over a period of 6 months (0, 1-2, 4-6 months).

Individuals with occupational exposure to blood or other potentially infectious materials (OPIM) may be at risk of acquiring Hepatitis B virus (HBV) infection. These individuals are asked to sign a form (institution specific) to certify that they have been given the opportunity to be

vaccinated against the Hepatitis B virus at no charge, if they have not already. It is important to note that many individuals receive this vaccine as part of their routine vaccinations.

**3) On-site blood-borne pathogen training checklist**

Students and instructors are provided with training inside the cell culture facility so that they can be familiar with specific work practices, the location of personal protective equipment (PPE) and eyewash facilities, biohazards waste handling, and disinfection/spill responses. At the end of the training, trainees complete and sign a verification of training certifying that the site-specific information was reviewed and understood.

**Appendix 2: Passaging HeLa Cells Student Protocol**

**Protocol:**

1. As starting material, use HeLa cells growing on a 100 mm dish at ~50-80% confluency. [Be sure to label your plates with date, initials, cell type, and passage number]
2. Aspirate spent media.
3. Rinse cells gently and carefully two times with room temperature PBS (~3 mL/rinse).
4. Add 1 mL warm 0.25% Trypsin/EDTA to cells
5. Incubate for 5 min at 37°C incubator
6. Add warm media (1 mL) to cells and pipette up and down to break up cell clumps.
7. Transfer the desired amount of cell suspension to seed growth in new 100 mm dish (1/10) recommended
8. Add 10 mL of DMEM 10% FBS to the new plate, gently swirl to mix and spread contents.
9. Place in 37°C incubator, track growth daily using the inverted microscope, you will need to passage again when confluence reaches ~50-80%.

**Appendix 3: Clonogenic Assay Student Protocols**

**HeLa Cell Colony Formation (Clonogenic) Assay on 6-well plates (Part I)**

**Protocol:**

1. As a starting material, use HeLa cells growing on a 100 mm dish at ~50-80% confluency.

   [Note: your group probably has more than one plate to use for this, select the plate that is closest to 80% confluency; **preparing one plate per group** should be enough for all members of the group to ultimately set up one colony formation assay (one 6-well plate per student)]

2. Aspirate spent media.

3. Rinse cells gently and carefully two times with PBS at room temperature (~3 mL each wash).

4. Add 1 mL warm 0.25% Trypsin/EDTA to cells.

5. Incubate for 5 min at 37°C incubator.

6. Add warm media (1 mL) to cells and pipette up and down to break up cell clumps.

7. Transfer ~2 mL of cell suspension into a 15 mL conical tube.

8. Measure the cell concentration (cell/microliter) using one of the chambers in a Kova Glasstic Slide (Kova International Cat. No. 87144) and a microscope.

To count cells:

Step 1- Using a micropipette, place 10 μL (undiluted harvested sample) into a blank counting chamber

Step 2- Using a microscope and using the grid as a focusing guide, find cells on small squares to count.

Step 3- Determine the average number of cells per small grid or square (count at least 7-10 small squares).

Step 4- Multiply the calculated average by 90. That is your cell concentration in cells/microliter.

9. Dilute your original stock cell suspension to a final cell concentration of 10 cells per microliter.

10. Into each of the wells of a 6-well plate, pipette 10 μL (100 cells) of your dilute cell suspension.

11. Using a pipette aid, pipette 3 mL DMEM with 5% FBS into each of the wells in top row.

**Note:** Make sure the bottoms of the wells are completely covered with media by gently rocking the plate. Be sure not to get media splatters on the lid during the process.

12. Using a pipette aid, pipette 3 mL DMEM with 10% FBS into each of the wells in bottom row.

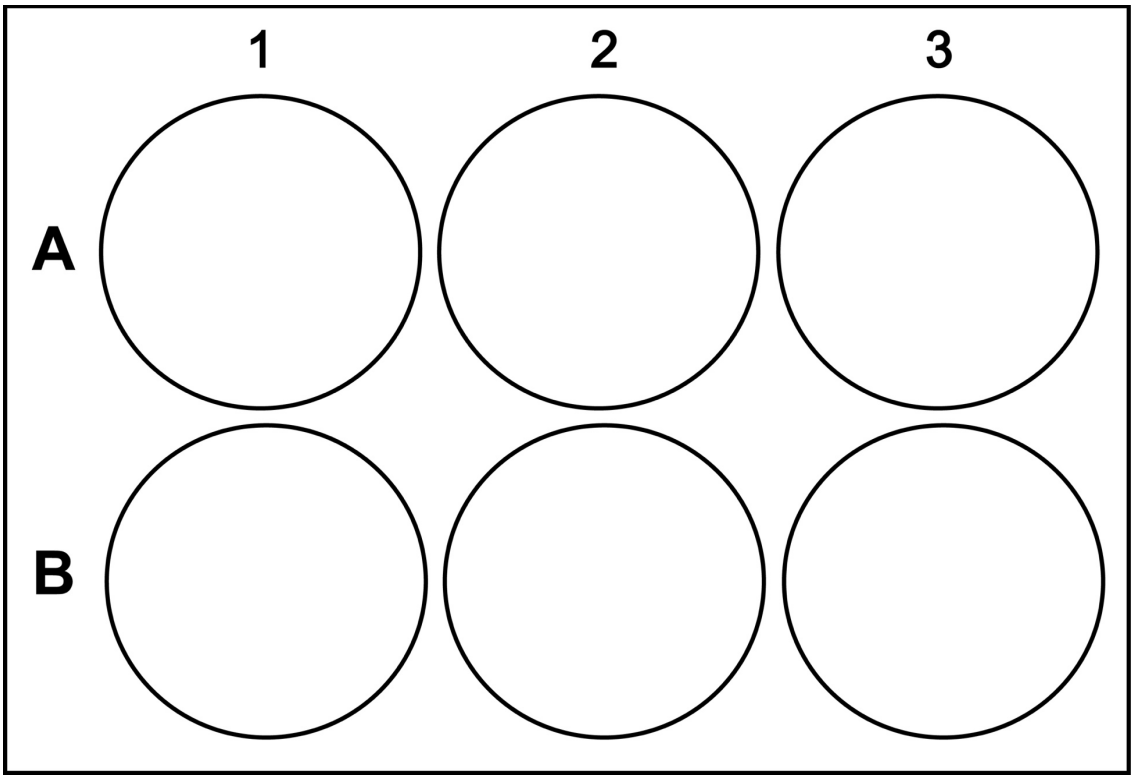
**Note:** Make sure the bottoms of the wells are completely covered with media by gently rocking the plate. Be sure not to get media splatters on the lid during the process.

13. Place your plate in the 37°C incubator and leave undisturbed for a whole week, until our next lab period.

**Note:** Place the plate deep inside the incubator to prevent disturbing of the plates which can happen when people grab their dishes or plates to check their cells. You do not need to check on your 6-well plate, just let the colonies grow undisturbed for a full week until our next lab period.

**HeLa Cell Colony Formation (Clonogenic) Assay on 6-well plates (Part II)**

**As a reminder, identify the experimental conditions of each of the wells on your 6-well plate:**




**Protocol:**

1. Aspirate the media out of each well (you do not have to switch pipette tips in between).

2. You will be using Richard-Allan Scientific Three-Step Stain Set (Thermo Fisher Cat. No. 3300) to fix and stain cell colonies.

3. Add 0.5 mL of **Fixative Solution** into each well of your 6-well plate.

4. Incubate at room temperature for 2 minutes.

5. Aspirate the solution out of each well (you do not have to switch pipette tips in between).

6. Add 0.5 mL of **Solution A (eosin Y dye, stains cytoplasm)** into each well of your 6-well plate.

7. Incubate at room temperature for 2 minutes.

8. Aspirate the solution out of each well (you do not have to switch pipette tips in between).

9. Add 0.5 mL of **Solution B (methylene blue/azure A dye, stains DNA i.e. nucleus)** into each well of your 6-well plate.

10. Incubate at room temperature for 2 minutes.

11. Aspirate the solution out of each of the wells (you do not have to switch pipette tips in between).

12. Rinse each well gently with deionized water using squirt bottle.

13. View colonies and take images as desired to record your results.

14. Record colony counts and perform appropriate statistics as desired.