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Title: Colony-Forming Unit Assay Adapted for Patient-Derived Bone Marrow and Peripheral Blood Mononuclear Cells

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Author Questionnaire

1. **Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done!**
3. **Filming location:** Will the filming need to take place in multiple locations? **Yes, Same building, same floor**
4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 14

Number of Shots: 52

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Aditi Shastri:** Our research investigates how genetic, epigenetic, and environmental stressors can drive hematopoietic stem cells toward leukemic transformation.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Mariapia Riso:** This CFU assay protocol provides a functional measure of therapeutic response in preclinical models of leukemia.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Samarpana Chakraborty:** This assay measures the differentiation capacities of human or murine-derived HSCs into erythroid or myeloid lineages in response to treatment.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Prachiben P. Patel:** Cells harvested from this assay can be used for several downstream analyses, such as flow cytometry, RT-qPCR, and western blotting.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Samarpana Chakraborty:** This assay can help in the identification of promising therapeutic candidates for further preclinical testing, and eventually for translation into the clinic.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. Aditi Shastri, Principal Investigator and Hematologist/Oncologist, Albert Einstein College of Medicine/Montefiore: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.7. Aditi Shastri, Principal Investigator and Hematologist/Oncologist, Albert Einstein College of Medicine/Montefiore: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at Albert Einstein College of Medicine and Montefiore Medical Center.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Cell Preparation and Viability Assessment of Patient-Derived Bone Marrow Cells or PBMC

Demonstrator: Mariapia Riso

2.1. To begin, warm Iscove's Modified Dulbecco's Medium, or IMDM (*I-M-D-M*), fetal bovine serum, or FBS (*F-B-S*), and penicillin-streptomycin antibiotics to 37 degrees Celsius for at least 30 minutes [1]. Next, warm the semisolid media to room temperature for at least 30 minutes after thawing it overnight at 4 degrees Celsius [2]. Prepare a waste beaker containing 10% bleach solution to dispose of contaminated pipette tips and serological pipettes [3]. Thaw frozen bone marrow cells isolated through density gradient centrifugation methods, in a 37 degrees Celsius water bath [4] and immediately place them on ice [5].

2.1.1. WIDE: Talent placing IMDM media, fetal bovine serum, and penicillin-streptomycin antibiotics in a warming area set to 37 degrees Celsius.

***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Talent placing the semisolid media bottle at room temperature.

2.1.3. Talent preparing a waste beaker containing 10% bleach solution.

2.1.4. Talent thawing frozen cell samples in a water bath.

2.1.5. Talent transferring the thawed cells onto ice.

2.2. Suspend the cells in 5 milliliters [1] of complete growth media [2]. Use acridine orange and propidium iodide dyes with an automated cell counter to determine the cell concentration and viability [3]. Centrifuge the cells at 350 g for 5 minutes at room temperature to pellet the cells [4].

2.2.1. Talent resuspending cells in complete growth media.

2.2.2. TEXT ON A PLAIN BACKGROUND

Complete Growth Media

- IMDM
- 4% FBS
- 1% Penicillin-Streptomycin

2.2.3. Talent loading stained cells into an automated cell counter.

2.2.4. Talent placing the sample into a centrifuge.

2.3. After discarding the supernatant, resuspend the cells in 5 milliliters of sterile Dulbecco's

phosphate-buffered saline, or DPBS (*D-P-B-S*) [1]. Centrifuge again to wash the cells [2]. Following the removal of DPBS, suspend the cells in complete growth media to achieve 100,000 cells per well in a 6-well plate [3].

- 2.3.1. Talent resuspending the pellet in Dulbecco's phosphate-buffered saline.
- 2.3.2. Talent placing the tube into a centrifuge.
- 2.3.3. Talent adjusting the cell concentration in complete growth media

3. Drug Treatment and Plating of Drug-treated Cell Suspension

3.1. On the day of plating, thaw [1] the prepared drug aliquots on ice [2]. Vortex to mix, then, spin down the aliquots in a tabletop mini centrifuge for 30 seconds at maximum speed [3].

3.1.1. Talent thawing the drug aliquot on ice.

3.1.2. TEXT ON A PLAIN BACKGROUND

Drug Treatments

- 10 μ M Pyrimethamine
- 4 μ M Venetoclax
- Combination of 10 μ M Pyrimethamine and 4 μ M Venetoclax
- Vehicle Control or DMSO

3.1.3. Talent placing the aliquot into a tabletop mini centrifuge. **Note:** This shot also included vortexing the drug aliquots before centrifuging, narration is also updated accordingly.

3.2. Vortex the semisolid media vigorously until completely homogenized [1]. Leave the media undisturbed in the hood at room temperature for 30 minutes to allow bubbles to rise [2]. Attach a blunt-end 16-gauge needle to a 3-milliliter syringe [3]. Use the syringe to aliquot 3 milliliters of semisolid media into sterile 15-milliliter conical tubes, setting aside for 30 minutes [4]. Next, add drug treatments, not exceeding 10 microliters, to each aliquot of semi-solid media [5].

3.2.1. Talent vortexing the semisolid media bottle.

3.2.2. Talent placing the media bottle undisturbed in the hood.

3.2.3. Talent attaching a blunt-end 16-gauge needle to a syringe.

3.2.4. Talent dispensing semisolid media into sterile conical tubes.

3.2.5. Talent adding drug to the semisolid media aliquots. **Author's note:** This shot also included a close-up shot.

- 3.3. After vortexing for complete drug incorporation, add 200,000 cells in 100 microliters of growth media to each 3-milliliter aliquot of semisolid media [1]. Vortex in short bursts until the mixture is homogenized [2]. Drop another 16-gauge needle and a 3-milliliter syringe into the cell mixture, leaving it undisturbed for 30 minutes [3].
 - 3.3.1. Talent adding cells to the semisolid media.
 - 3.3.2. Talent placing the cell and drug-media mixture on the vortex for mixing.
 - 3.3.3. Talent placing the needle and syringe into the mixture. **Author's note: This shot also included a close-up shot.**
- 3.4. Use the submerged needle and syringe to aspirate the cell and drug mixture without introducing air bubbles [1]. After allowing the mixture to settle for 3 to 5 minutes at the bottom of the tube, aspirate the remaining volume, avoiding air bubbles [2]. Add the mixture dropwise into 2 wells of an outer column of a sterile 6-well plate [3]. Gently tilt the plate in circular motions to spread the mixture evenly across the wells [4].
 - 3.4.1. Talent aspirating the mixture with the submerged syringe.
 - 3.4.2. Talent aspirating the remaining mixture collected at the bottom from the tube walls.
 - 3.4.3. Talent dispensing the mixture into two wells.
 - 3.4.4. Talent tilting the plate to distribute the mixture evenly.
- 3.5. Add 5 milliliters of sterile water to each well in the center column [1]. Incubate the plate at 37 degrees Celsius with 5% carbon dioxide [2].
 - 3.5.1. Talent adding sterile water to the center wells.
 - 3.5.2. Talent placing the plate into an incubator.

4. Colony Imaging and Counting

- 4.1. Insert a light diffuser into the top lens of the microscope apparatus [1]. In the imaging system interface, under the capture tab, set the objective to 4X magnification [2]. Select the light source as **Trans** to switch the microscope to **brightfield mode** [3]. After the microscope light icon turns on, adjust the intensity using the coarse slider to a range of 0.03 [4]. Next, click on the autofocus icon and adjust fine focus controls to obtain a sharp image, as required [5].
 - 4.1.1. Talent inserting a light diffuser into the microscope.
 - 4.1.2. SCREEN: 71526_screenshot_1.mp4. 00:00–00:06
 - 4.1.3. SCREEN: 71526_screenshot_1.mp4. 00:08–00:14
 - 4.1.4. SCREEN: 71526_screenshot_1.mp4. 00:18–00:21; 00:24–00:28

4.1.5. SCREEN: 71526_screenshot_1.mp4. 00:30–00:33; 00:36–00:39; 00:40–00:45

- 4.2. Under the automate tab showing the image capture pattern, set the appropriate area and field acquisition orders, select the stitch images option, and then click on Done [1]. In the **Image Save Settings** menu, click **Review**, then select the option to tile or merge raw images from each well [2]. Under the Settings tab, general menu, select the option to invert images horizontally and vertically [3]. Configure autofocus to refocus in each well, and click on **Done** [4]. Return to the automate tab and run the automated plate scan and manually count total, myeloid, and erythroid colonies using the merged images [5].

4.2.1. SCREEN: 71526_screenshot_2.mp4. 00:02–00:11; 00:13–00:14

4.2.2. SCREEN: 71526_screenshot_2.mp4. 00:14– 00:22

4.2.3. SCREEN: 71526_screenshot_2.mp4. 00:29– 00:33; 00:34–00:37

4.2.4. SCREEN: 71526_screenshot_2.mp4. 00:39–00:42

4.2.5. SCREEN: 71526_screenshot_2.mp4. 00:44–00:48; 00:51–00:55; 01:52–01:54

5. Cell Harvesting and Freezing for Downstream Flow Cytometric Analysis

- 5.1. Add 5 milliliters of warmed FACS buffer to the surface of each well [1]. Incubate the plates at 37 degrees Celsius for at least 30 minutes to dissolve the semisolid media [2]. Thoroughly resuspend the cells using a 5-milliliter pipette [3]. Transfer the cell suspension to a sterile 50 milliliter conical tube [4]. After washing each well 3 times with warmed FACS buffer, transfer the wash volume to the same tube [5].

5.1.1. Talent adding warmed FACS buffer to the wells.

5.1.2. Talent placing the plate into an incubator.

5.1.3. Talent resuspending cells with a pipette.

5.1.4. Talent transferring cells into a sterile conical tube.

5.1.5. Talent collecting the wash solution into same 50 mL tube.

- 5.2. Followed by vortexing in 4 to 5 short bursts, centrifuge the suspension at 400 *g* for 10 minutes at room temperature [1]. ~~Incubate at 37 degrees Celsius for 10 to 15 minutes to dissolve remaining media [2]. Pellet the cells again [3].~~

5.2.1. Talent placing the tube into a centrifuge.

5.2.2. ~~Talent placing the tube into an incubator.~~ **Note: Shots 5.2.2 and 5.2.3 and their corresponding narrations were omitted.**

5.2.3. ~~Talent preparing the sample for a second centrifugation.~~

- 5.3. After discarding the supernatant, resuspend the cells in 10 milliliters of FACS buffer [1]. Centrifuge at 400 *g* for 10 minutes at room temperature to pellet the cells [2].

- 5.3.1. Talent resuspending cells in FACS buffer.
- 5.3.2. Talent placing the sample into a centrifuge.
- 5.4. Resuspend the cells in freezing media [1]. Aliquot 3 million cells into each cryovial [2]. Place the cryovials into a controlled-rate freezing container at minus 80 degrees Celsius for 24 hours, followed by transfer to liquid nitrogen storage [3].
 - 5.4.1. Talent resuspending cells in freezing media.
 - 5.4.2. Talent dispensing cells into cryovials.
 - 5.4.3. Talent placing cryovials into a controlled-rate freezing container.

Results

6. Results

6.1. Enumerated colony counts from a patient-derived sample at 13 days post-plating shows fewer overall colonies in the pyrimethamine-only treatment [1] and combination treatment with venetoclax [2].

6.1.1. LAB MEDIA: Figure 3B. *Video Editor: Please emphasize the “Pyr” and “Combo” bars in the top “total counts” graph.*

6.2. Representative images show that myeloid colonies appear as dispersed clusters without clearly defined edges and are typically more translucent and less pigmented [1]. Erythroid colonies appear as more defined, dense colonies or colony clusters with red or dark pigmentation and clear, defined edges [2]. Overlapping colonies are categorized as mixed, multipotent progenitor colonies [3].

6.2.1. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the spread-out cluster in the left panel.*

6.2.2. LAB MEDIA: Figure 4B. *Video Editor: Please emphasize the dense, red colored cluster in the left panel.*

6.2.3. LAB MEDIA: Figure 4C. *Video Editor: Please highlight both less dense and highly dense clusters in the center panel.*

Pronunciation Guide:

1. Viability

Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>

IPA: /ˌvaɪ.əˈbɪl.ə.ti/

Phonetic Spelling: vy·uh·bil·uh·tee

2. Patient-derived

Pronunciation link: <https://www.merriam-webster.com/dictionary/patient>

IPA: /ˈpeɪ.jənt/; /dɪˈraɪvd/

Phonetic Spelling: pay·shuhnt; dih·ryvd

3. Bone Marrow

Pronunciation link: <https://www.merriam-webster.com/dictionary/bone%20marrow>

IPA: /ˈboʊn ˌmer.oʊ/

Phonetic Spelling: bohn·mair·oh

4. PBMC

Pronunciation link: No confirmed link found

IPA: No confirmed IPA found

Phonetic Spelling: pee·bee·em·see

5. Iscove's
Pronunciation link: No confirmed link found
IPA: No confirmed IPA found
Phonetic Spelling: iss·kohvz
6. Dulbecco's
Pronunciation link: No confirmed link found
IPA: No confirmed IPA found
Phonetic Spelling: dul·bek·ohz
7. IMDM
Pronunciation link: No confirmed link found
IPA: No confirmed IPA found
Phonetic Spelling: eye·em·dee·em
8. Fetal
Pronunciation link: <https://www.merriam-webster.com/dictionary/fetal>
IPA: /'fi:təl/
Phonetic Spelling: fee·tuhl
9. Bovine
Pronunciation link: <https://www.merriam-webster.com/dictionary/bovine>
IPA: /'boʊ.vain/
Phonetic Spelling: boh·vyn
10. Penicillin-streptomycin
Pronunciation link: <https://www.merriam-webster.com/dictionary/penicillin>
IPA: /,pen.ə'sɪl.ɪn/; /,strep.tə'maɪ.sɪn/
Phonetic Spelling: pen·uh·sil·in; strep·tuh·my·sin
11. Semisolid
Pronunciation link: <https://www.merriam-webster.com/dictionary/semisolid>
IPA: /,sem.i'sɑː.lɪd/
Phonetic Spelling: sem·ee·saa·lid
12. Thawing
Pronunciation link: <https://www.merriam-webster.com/dictionary/thaw>
IPA: /'θɔː.ɪŋ/
Phonetic Spelling: thaw·ing
13. Density
Pronunciation link: <https://www.merriam-webster.com/dictionary/density>
IPA: /'den.sə.ti/
Phonetic Spelling: den·suh·tee
14. Gradient
Pronunciation link: <https://www.merriam-webster.com/dictionary/gradient>
IPA: /'ɡreɪ.di.ənt/
Phonetic Spelling: gray·dee·uhnt
15. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>

IPA: /ˌsen.trə.fjuːˈgeɪ.jən/

Phonetic Spelling: sen·truh·fyoo·gay·shuhn

16. Acridine

Pronunciation link: <https://www.howtopronounce.com/acridine>

IPA: /ˈæk.rɪ.diːn/

Phonetic Spelling: ak·ruh·deen

17. Propidium

Pronunciation link: <https://www.howtopronounce.com/propidium>

IPA: /prɒˈpɪd.i.əm/

Phonetic Spelling: proh·pid·ee·uhm

18. Iodide

Pronunciation link: <https://www.merriam-webster.com/dictionary/iodide>

IPA: /ˈaɪ.əˌdaɪd/

Phonetic Spelling: eye·uh·dyd

19. Automated

Pronunciation link: <https://www.merriam-webster.com/dictionary/automated>

IPA: /ˈɔː.təˌmeɪ.tɪd/

Phonetic Spelling: aw·tuh·may·tuhd

20. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /ˌsuː.pəˈneɪ.tənt/

Phonetic Spelling: soo·per·nay·tuht

21. Phosphate-buffered

Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>

IPA: /ˈfɔːs.fert/; /ˈbʌf.əd/

Phonetic Spelling: fos·fayt; buf·erd

22. DPBS

Pronunciation link: No confirmed link found

IPA: No confirmed IPA found

Phonetic Spelling: dee·pee·bee·ess

23. Aliquots

Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>

IPA: /ˈæl.əˌkwɑːt/

Phonetic Spelling: al·uh·kwaht

24. Pyrimethamine

Pronunciation link: <https://www.merriam-webster.com/dictionary/pyrimethamine>

IPA: /ˌpɪr.əˈmeθ.ə.miːn/

Phonetic Spelling: peer·uh·meth·uh·meen

25. Venetoclax

Pronunciation link: <https://www.howtopronounce.com/venetoclax>

IPA: /vəˌnetəˈklæks/

Phonetic Spelling: vuh·net·uh·klaks

26. DMSO
Pronunciation link: No confirmed link found
IPA: No confirmed IPA found
Phonetic Spelling: dee·em·ess·oh
27. Homogenized
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /hə'mɑː.dʒə.naɪzd/
Phonetic Spelling: huh·mah·juh·nyzd
28. Blunt-end
Pronunciation link: <https://www.merriam-webster.com/dictionary/blunt-end>
IPA: /'blʌnt ˌend/
Phonetic Spelling: blunt·end
29. Conical
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>
IPA: /'kɑː.nɪ.kəl/
Phonetic Spelling: kon·ih·kuhl
30. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪn.kjəˌbeɪt/
Phonetic Spelling: in·kyuh·bayt
31. Carbon dioxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/carbon%20dioxide>
IPA: /'kɑːr.bən daɪ'ɑːk.saɪd/
Phonetic Spelling: kar·buhn dye·ok·syd
32. Diffuser
Pronunciation link: <https://www.merriam-webster.com/dictionary/diffuser>
IPA: /dɪ'fjuː.zə/
Phonetic Spelling: dih·fyoo·zer
33. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>
IPA: /'maɪ.krəˌskoʊp/
Phonetic Spelling: my·kruh·skohp
34. Apparatus
Pronunciation link: <https://www.merriam-webster.com/dictionary/apparatus>
IPA: /ˌæp.ə'ræʧ.əs/
Phonetic Spelling: ap·uh·rat·uhs
35. Magnification
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnification>
IPA: /ˌmæɡ.nə.fə'keɪ.jən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn
36. Brightfield
Pronunciation link: No confirmed link found

IPA: No confirmed IPA found

Phonetic Spelling: bryt·feeld

37. Autofocus

Pronunciation link: <https://www.merriam-webster.com/dictionary/autofocus>

IPA: /ˌɔː.tʊʃˈfoʊ.kəs/

Phonetic Spelling: aw·toh·foh·kuhs

38. Automate

Pronunciation link: <https://www.merriam-webster.com/dictionary/automate>

IPA: /ˈɔː.təˌmeɪt/

Phonetic Spelling: aw·tuh·mayt

39. Myeloid

Pronunciation link: <https://www.merriam-webster.com/dictionary/myeloid>

IPA: /ˈmaɪ.ə.lɔɪd/

Phonetic Spelling: my·uh·loyd

40. Erythroid

Pronunciation link: <https://www.merriam-webster.com/dictionary/erythroid>

IPA: /ˈɪrɪθ.rɔɪd/

Phonetic Spelling: ih·rith·royd

41. Downstream

Pronunciation link: <https://www.merriam-webster.com/dictionary/downstream>

IPA: /ˌdaʊnˈstriːm/

Phonetic Spelling: down·streem

42. Cytometric

Pronunciation link: <https://www.merriam-webster.com/dictionary/cytometry>

IPA: /ˌsaɪ.təˈmet.rɪk/

Phonetic Spelling: sy·tuh·met·rik

43. FACS

Pronunciation link: No confirmed link found

IPA: No confirmed IPA found

Phonetic Spelling: faks

44. Cryovial

Pronunciation link: <https://www.howtopronounce.com/cryovial>

IPA: /ˈkraɪ.ooˌvaɪ.əl/

Phonetic Spelling: kry·oh·vy·uhl

45. Cryovials

Pronunciation link: <https://www.howtopronounce.com/cryovial>

IPA: /ˈkraɪ.ooˌvaɪ.əlz/

Phonetic Spelling: kry·oh·vy·uhlz

46. Controlled-rate

Pronunciation link: <https://www.merriam-webster.com/dictionary/control>

IPA: /kənˈtroʊld reɪt/

Phonetic Spelling: kuhn·trohld·rayt