

Submission ID #: 67956

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Project Page Link: <https://review.jove.com/account/file-uploader?src=20726628>

Title: A Streamlined, Label-Free Real-Time 50% Tissue Culture Infectious Dose (TCID₅₀) Assay using Impedance for Automated Viral Titer Quantification

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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? **No**
- 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. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 42

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Xiaoyu Zhang:** The scope of my research was to develop a method accelerating viral quantification, reducing hands-on time, enabling high-throughput CPE tracking.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What research gap are you addressing with your protocol?~~

- 1.2. **Xiaoyu Zhang:** This protocol addresses the lack of a reliable, simple, fast, safe, and highly consistent method compared with conventional viral quantification approaches.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Xiaoyu Zhang:** Our protocol offers fully automated, label-free, real-time, and objective viral quantification using impedance-based TCID50 analysis with integrated virology software.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

- 1.4. **Xiaoyu Zhang:** Current challenges include reliance on staining with toxic reagents in conventional TCID50 assays and subjectivity and analyst variability in label-free imaging methods.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Permissive Cell Seeding into a Biosensor Plate

Demonstrator: Jing Zhang

- 2.1. To begin, measure the impedance background of a biosensor plate before cell seeding [1]. Use a multichannel pipette to carefully transfer 50 microliters of prewarmed media into each well [2]. Use the reverse pipetting technique to minimize bubble formation and ensure consistent volume dispensation [3].
 - 2.1.1. WIDE: Talent holding an empty biosensor plate.
 - 2.1.2. Talent dispensing prewarmed media into all wells of the biosensor plate using a multichannel pipette.
 - 2.1.3. Shot of the reverse pipetting technique.
- 2.2. Place the plate into the plate station [1]. Then launch the system's software and select the **Virology** module [2].
 - 2.2.1. Talent placing the biosensor plate into the plate station.
 - 2.2.2. SCREEN: [67956 2.2.2.mp4](#) 00:03-00:08, 00:15-00:19
- 2.3. Enter relevant notes in the **Exp Notes** (*Experiment-Note*) tab [1] and input cell information including cell name and cell number in the **Cell** subtab of the **Layout** tab after selecting the corresponding wells [2].
 - 2.3.1. SCREEN: [67956 2.3.1.mp4](#) 00:03-00:13
 - 2.3.2. SCREEN: [67956 2.3.2.mp4](#) 00:02-00:16
- 2.4. Next, on the **Schedule** tab, click **Add a Step** to create **Step 1** designated for plate background measurement [1-TXT]. ~~Initiate recording by clicking **Start** [2].~~
 - 2.4.1. SCREEN: [67956 2.4.1-&2.4.2.mp4](#). 00:01-00:09
TXT: Click Start to initiate recording
 - ~~2.4.2. SCREEN: [67956 2.4.1-&2.4.2.mp4](#).~~
- 2.5. Remove the plate from the plate station [1] and place it in the laminar hood for cell seeding [2].
 - 2.5.1. Talent removing the plate from the plate station.
 - 2.5.2. Talent transferring it to the laminar hood.
- 2.6. Add 2 microliters of trypsin to detach the permissive cells from the T75 (*T-Seventy-five*)

flask [1]. After counting the cells, adjust the cell suspension to the desired concentration [2].

2.6.1. Talent adding 2 μ L trypsin to the T75 flask to detach the cells.

2.6.2. Talent adjusting the cell suspension concentration.

2.7. Pipette 100 microliters of the cell suspension to each well of the biosensor plate [1-TXT]. After incubating it for 30 minutes at room temperature, transfer the plate into the plate station inside incubator at 37 degrees Celsius with 5 percent carbon dioxide [2].

2.7.1. Talent dispensing 100 μ L of the cell suspension into each well of the biosensor plate. **TXT: TXT: Cells: HEK293A cells or MDCK cells**

2.7.2. Talent placing the plate station into the incubator.

2.8. Navigate to the **Schedule** tab and add **Step 2** using the default recording settings of 100 sweeps and a 15-minute interval [1].

2.8.1. SCREEN: [67956_2.8.1.mp4](#). 00:00-end

3. Virus Serial Dilution, Inoculation, and Real-Time TCID50 Analysis

3.1. To prepare virus serial dilutions, calculate the total volume of medium needed for dilution [1-TXT]. Warm the medium in a 37-degree Celsius water bath for 30 minutes [2].

3.1.1. Shot of the required volume of medium. **TXT: Medium: DMEM + 2% FBS + 1% penicillin /streptomycin**

3.1.2. Talent placing the medium into a water bath to warm.

3.2. Rapidly thaw an aliquoted virus stock vial in the water bath for 1 to 2 minutes until all ice crystals have melted [1].

3.2.1. Talent thawing the virus stock vial in the water bath.

3.3. Transfer 20 microliters of virus stock into a 1.5-milliliter microcentrifuge tube containing 180 microliters of prewarmed media [1]. Pipette up and down to thoroughly mix the virus with the medium [2].

3.3.1. Talent transferring virus stock into the microcentrifuge tube containing media.

3.3.2. Talent pipetting the mixture up and down to mix thoroughly.

3.4. To equilibrate the virus dilutions, incubate the diluted virus samples at 37 degrees Celsius with 5 percent carbon dioxide for 20 minutes prior to inoculation [1].

3.4.1. Talent placing virus dilution tubes into the incubator.

3.5. Next, on the **Layout** tab of the software, select the wells designated for viral titer treatment [1]. ~~Click the Treatment subtab and~~ Enter the initial **dilution** value of 0.001 [2].

- 3.5.1. SCREEN: [67596 3.5.1-3.6.2.mp4](#). 00:01-00:06
- 3.5.2. SCREEN: [67596 3.5.1-3.6.2.mp4](#). 00:07-00:13
- 3.6. Enter **10** as the **dilution factor** and select the **direction** of dilution from left to right **[1]**. Then enter the virus **inoculation volume** and click **Apply** to save the treatment information **[2]**.
- 3.6.1. SCREEN: [67596 3.5.1-3.6.2.mp4](#). 00:14-00:18
- 3.6.2. SCREEN: [67596 3.5.1-3.6.2.mp4](#). 00:19-00:24
- 3.7. Assign negative control wells by selecting the appropriate wells and checking **Negative Ctrl** (*negative-control*) under **Well Type** in the **Treatment** tab **[1]**.
- 3.7.1. SCREEN: [67956 3.7.1.mp4](#). 00:01-00:08
- 3.8. To inoculate the virus, click **Pause** in the software to stop data acquisition **[1]**. Remove the plate from the plate station and place it in the laminar hood **[2]**.
- 3.8.1. SCREEN: [67956 3.8.1.mp4](#). 00:00-end
- 3.8.2. Talent transferring the plate from the plate station to the laminar hood.
- 3.9. Using a multichannel pipette, carefully remove the culture media from each well **[1]**. Gently add 50 microliters of the virus dilution onto the top of the cell monolayer, avoiding disruption of the cells **[2]**.
- 3.9.1. Talent aspirating culture media from the wells using a multichannel pipette.
- 3.9.2. Talent gently adding virus dilutions to the wells.
- 3.10. Place the plate back into the plate station **[1]**. On the **Schedule** tab, add **Step 3** using the default recording settings to resume impedance recording **[2]**.
- 3.10.1. Talent placing the plate back into the plate station.
- 3.10.2. SCREEN: [67596 3.10.2.mp4](#). 00:00-end
- 3.11. After 2 hours of virus inoculation, pause the recording **[1]** and remove the plate from the plate station to the laminar hood **[2]**.
- 3.11.1. SCREEN: [67956 3.11.1.mp4](#). 00:00-end
- 3.11.2. Shot of plate being transferred to the laminar hood.
- 3.12. With a multichannel pipette, slowly add 150 microliters of prewarmed medium to each well, avoiding disruption of the cell monolayer **[1]**. Return the plate to the plate station **[2]** and resume recording by clicking **Start** **[3]**.
- 3.12.1. Talent adding prewarmed medium gently to each well.
- 3.12.2. Shot of plate in the plate station
- 3.12.3. SCREEN: [67956 3.12.3.mp4](#). 00:00-end

3.13. To calculate the tissue culture infectious dose 50, navigate to the **Data Analysis** tab and **add all assay wells** to the plot chart [1]. In the **Parameter** section, select **TCID50 (T-C-I-D-Fifty)** from the dropdown list to initiate calculation [2].

3.13.1. SCREEN: [67956 3.13.1-&3.13.2.mp4](#). 00:00-00:05

3.13.2. SCREEN: [67956 3.13.1-&3.13.2.mp4](#). 00:06-00:12

3.14. Choose **Bar Chart** to calculate TCID50 at a specific time point [1] or select **Time Dependent TCID50** to generate a TCID50 time course over the desired period [2].

3.14.1. SCREEN: [67956 3.14.1.mp4](#). 00:02-00:09

3.14.2. SCREEN: [67956 3.14.2.mp4](#). 00:02-end

Results

4. Results

- 4.1. In uninfected HEK293A (*H-E-K-Two-Ninety-Three*) cells, the Normalized Cell Index increased over the first 48 hours post-inoculation and then plateaued, indicating continued cell proliferation followed by confluence [1].
 - 4.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the black trace*
- 4.2. In Adv-GFP (*Adenoviral-G-F-P*)–infected HEK293A cells, the Normalized Cell Index dropped below the uninfected control approximately 50 hours post-inoculation and declined to zero by 100 hours, indicating virus-induced cytopathic effects [1].
 - 4.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the red trace decreasing from around 50 hours until reaching zero.*
- 4.3. Viral green fluorescent protein expression increased inversely as the Normalized Cell Index decreased during the first 100 hours post-inoculation in Adv-GFP–infected HEK293A cells [1].
 - 4.3.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the green trace representing integrated green intensity increasing over time.*
- 4.4. Live-cell imaging showed continued cell growth during the first 48 hours post-infection, followed by reduced confluency and increased green fluorescent protein signal as viral infection progressed [1], with loss of viable cells observed by approximately 100 hours [2].
 - 4.4.1. LAB MEDIA: Figure 2B. *Video editor: Highlight Brightfield and Brightfield + green images for 1-3*
 - 4.4.2. LAB MEDIA: Figure 2B. *Video editor: Highlight Brightfield and Brightfield + green images for 4-5*
- 4.5. Adv-GFP infection induced a dose-dependent decrease in the Normalized Cell Index, with higher virus concentrations causing earlier and steeper declines across replicate wells [1].
 - 4.5.1. LAB MEDIA: Figure 3A. *Video editor: Sequentially highlight the colored traces from 10^{-3} to 10^{-10}*
- 4.6. At a 10^{-8} dilution of Adv-GFP, the Normalized Cell Index exhibited high variability across replicate wells, indicating partial cytopathic effects [1].
 - 4.6.1. LAB MEDIA: Figure 3A. *Video editor: Show the pop up of 10^{-8} (right side) then highlight the light blue traces labeled 10^{-8}*
- 4.7. The Adv-GFP TCID₅₀ increased over time and reached a plateau by approximately 150 hours post-inoculation [1].

- 4.7.1. LAB MEDIA: Figure 4. *Video editor: Highlight the plotted points showing increasing values*
- 4.8. In MDCK cells infected with influenza A virus, impedance measurements showed dose-dependent changes in the Normalized Cell Index compared to uninfected controls [1].
- 4.8.1. LAB MEDIA: Figure 5A. *Video editor: Highlight multiple colored traces*

Pronunciation Guide:

TCID50

Pronunciation link: <https://www.merriam-webster.com/dictionary/TCID50>

IPA: /ˌtiːsiː.ərˈdiː ˈfɪfti/

Phonetic Spelling: tee-see-eye-dee fif-tee

Impedance

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

IPA: /ɪmˈpiːdəns/

Phonetic Spelling: im-pee-duhns

Quantification

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

IPA: /ˌkwɑːntəfəˈkeɪʃən/

Phonetic Spelling: kwahn-tuh-fuh-kay-shuhn

Permissive

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

IPA: /pərˈmɪsɪv/

Phonetic Spelling: per-miss-iv

Biosensor

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

IPA: /ˈbaɪoʊˌsɛnsər/

Phonetic Spelling: bye-oh-sen-ser

Dispensation

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

IPA: /ˌdɪspənˈseɪʃən/

Phonetic Spelling: dis-puhn-say-shuhn

Laminar

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

IPA: /ˈlæmənər/

Phonetic Spelling: lam-uh-ner

Trypsin

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

IPA: /ˈtrɪpsɪn/

Phonetic Spelling: trip-sin

Inoculation

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

IPA: /ɪˌnɔːkjəˈleɪʃən/

Phonetic Spelling: ih-nah-kyuh-lay-shuhn

Microcentrifuge

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

IPA: /ˌmaɪkrəʊˈsentrəˌfjuːdʒ/

Phonetic Spelling: my-kroh-sen-truh-fyooj

Aliquoted

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

IPA: /ˈælɪˌkwɑːt/

Phonetic Spelling: al-ih-kwot

Cytopathic

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

IPA: /ˌsaɪtəˈpæθɪk/

Phonetic Spelling: sy-tuh-path-ik

Adenoviral

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

IPA: /ˈædənoʊˌvaɪrəs/

Phonetic Spelling: ad-uh-noh-vy-ruhs

Confluence

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

IPA: /ˈkɑːnfluəns/

Phonetic Spelling: kon-floo-uhns

Proliferation

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

IPA: /prəˌlɪfəˈreɪʃən/

Phonetic Spelling: ɹpruh-lif-uh-ray-shuhn

Confluency

Pronunciation link: No confirmed link found

IPA: /ˈkɑːnfluənsi/

Phonetic Spelling: kon-floo-uhn-see

Influenza

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

IPA: /ˌɪnfluˈenzə/

Phonetic Spelling: in-floo-en-zuh

Variability

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

IPA: /ˌveriəˈbɪləti/

Phonetic Spelling: vair-ee-uh-bil-uh-tee