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Project Page Link: <https://review.jove.com/account/file-uploader?src=21713913>

Title: A Single Intravenous Dose of Indole-Producing Attenuated Brucella in NOD Mice: Assessment of Type 1 Diabetes Onset and Islet Immune Remodeling

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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, withing the same building. Bacteria preparation, culture preparation and IV injections will be done upstairs in designated bacteria room (3rd floor). All mice work and subsequent video will be conducted in TAMU animal vivarium (1st floor). Easily accessible via elevator.**

- 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: 26

Number of Shots: 42

Introduction

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

Authors: Answers are slightly edited in accordance with journal guidelines. Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

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

- 1.1. **Kaylee Delgado:** This research focuses on evaluating whether a single intravenous dose of metabolically engineered bacteria can prevent autoimmune type 1 diabetes.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Kaylee Delgado:** Current therapies require repeated dosing and fail to provide durable tolerance, while this protocol presents a single-dose, tissue-remodeling bacterial strategy.
 - 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. **Kaylee Delgado:** Using this approach, researchers can measure glycemic control, quantify preserved islet area, and map spatial immune remodeling within the pancreas.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Kaylee Delgado:** The most critical challenge is executing precise intravenous tail vein injections without anesthesia to ensure accurate bacterial dosing.
 - 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. **Kaylee Delgado**: Future studies can investigate specific mechanisms, focusing on whether indole acts directly on T cells or requires macrophage intermediaries.
 - 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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University

Protocol

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

2. Preparation of Bacterial Suspension

Demonstrator: Kaylee Delgado

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

- 2.1. To begin, obtain chloramphenicol-supplemented tryptic soy agar or TSA (*T-S-A*) plates less than or equal to 1 week old containing isolated bacterial colonies [1-TXT]. Using a sterile loop, pick 2 to 3 isolated colonies [2] and inoculate it into 10 milliliters of tryptic soy broth supplemented with chloramphenicol in a 15 milliliter tube [3]. Incubate at 37 degrees Celsius with shaking at 250 revolutions per minute under ambient carbon dioxide conditions [4].
 - 2.1.1. WIDE: Talent walking into the room with a tray containing TSA plates. **TXT: Bacterial strain: BmΔvjbR::tnaA**
 - 2.1.2. Talent picking colonies using a sterile loop.
 - 2.1.3. Talent transferring colonies into a tube containing broth. **Videographer's NOTE: 2.1.2 and 2.1.3 are combined in one shot.**
 - 2.1.4. Talent placing the tube into a shaking incubator.
- 2.2. Blank the spectrophotometer using sterile TSB supplemented with chloramphenicol [1]. Measure optical density at 600 nanometers every 2 to 3 hours and continue incubation until optical density reaches 2.0 plus or minus 0.2 [2].
 - 2.2.1. Talent inserting a blank sample into the spectrophotometer and calibrating it.
 - 2.2.2. Talent measuring optical density using the spectrophotometer. **Videographer's NOTE: 2.2.1 and 2.2.2 are combined in one shot.**
- 2.3. Estimate bacterial concentration [1-TXT].
 - 2.3.1. Talent calculating the OD in a lab notebook. **TXT: OD₆₀₀ = 1 corresponds to 5 × 10⁹ CFU/mL**
- 2.4. Transfer the 10 milliliter culture to a sterile 15 milliliter conical tube [1]. Centrifuge at a relative centrifugal force of 3000 to 4000 for 10 minutes at room temperature [2]. Carefully aspirate and discard the supernatant without disturbing the pellet [3].
 - 2.4.1. Talent adding culture into a conical tube.

- 2.4.2. Talent placing the tube into a centrifuge.
- 2.4.3. Talent removing supernatant while leaving pellet intact.
- 2.5. Add 1 milliliter of sterile endotoxin free 1 times phosphate buffered saline or PBS (*P-B-S*) to the bacterial pellet [1]. Using a pipette, resuspend by pipetting 10 to 15 times until homogeneous with no visible clumps [2].
 - 2.5.1. Talent adding buffer to the pellet.
 - 2.5.2. Talent pipetting to resuspend the pellet.
- 2.6. Keep the bacterial suspension on ice in a closed tube and use the suspension for intravenous injections within 4 hours of preparation [1].
 - 2.6.1. Talent placing the tube on ice.

Videographer's NOTE: 2.5.1, 2.5.2 and 2.6.1 are combined in one shot.

3. Animal Housing and Pre-Procedure Preparation

- 3.1. Obtain female mice younger than 5 weeks of age and acclimate them for at least 7 days prior to experimentation [1-TXT].
 - 3.1.1. WIDE: Mice housed in cages in an animal facility. **TXT: Mouse strain: NOD ShiLtJ**
- 3.2. House up to five mice in each individually ventilated cage under specific pathogen-free conditions. [1].
 - 3.2.1. Shot of mice inside ventilated cages.
- 3.3. Provide bedding, enrichment, food, and water ad libitum [1]. Maintain temperature at 20 to 24 degrees Celsius [2], humidity at 40 to 60 percent, and a 12 hour light and dark cycle [3].
 - 3.3.1. Shot of cage setup with bedding and food.
 - 3.3.2. Close-up of temperature control panel displaying temperature 20-24°C.
 - 3.3.3. Close-up of an environmental monitor displaying humidity 40%-60%.

Videographer's NOTE: 3.3.1, 3.3.2 and 3.3.3 are combined in one shot.
- 3.4. Record baseline body weights of the mice [1].
 - 3.4.1. Talent weighing a mouse on a scale.
- 3.5. Randomize mice into treatment and control groups and assign unique identifiers using ear tag or tail tattoo and transfer the cages to procedure room [1].
 - 3.5.1. Talent tagging or marking mice. **TXT: Place the cages on heating pads at 37-40°C for 30 mins** NOTE: The removed step 3.6 is concisely added in narration and TXT.

- 3.6. Transfer cages to the procedure room at least 30 minutes prior to injection [1]. Place the cages on heating pads set to 37 to 40 degrees Celsius to promote tail vein dilation [2].
 - 3.6.1. Talent moving cages into procedure room.
 - 3.6.2. Talent placing cages on heating pads. NOTE: 3.6 was not filmed. For removed shots, VO is added in concise format in previous step.

4. Intravenous Administration via Lateral Tail Vein

- 4.1. Under sterile conditions, draw 100 microliters of bacterial suspension into 29 to 30 gauge insulin syringes [1] and label each syringe with the corresponding mouse identification [2]. Prepare control syringes with 100 microliters of sterile PBS [3].
 - 4.1.1. Talent filling syringes with bacterial suspension.
 - 4.1.2. Talent labeling the syringes with mouse ID.
 - 4.1.3. Talent filling control syringes with PBS.
- 4.2. Place each mouse in a restrainer allowing access to the tail [1].
 - 4.2.1. Talent securing mouse in restrainer.
- 4.3. Wipe the tail with 70 percent ethanol and let it air dry [1].
 - 4.3.1. Talent cleaning the mouse tail. Videographer's NOTE: 4.2.1 and 4.3.1 are combined in one shot.
- 4.4. Visualize the lateral tail veins under adequate lighting [1].
 - 4.4.1. Close-up of tail veins under light.
- 4.5. Hold the distal third of the tail with gentle tension [1]. Insert the assigned syringe needle bevel up at a shallow angle of 10 to 15 degrees into the lateral vein [2].
 - 4.5.1. Close-up of the talent holding the far end of the mouse tail.
 - 4.5.2. Close-up of needle being inserted into tail vein.
- 4.6. Inject the solution into each mouse slowly over 5–10 seconds, in a randomized order within a 2-hour window [1]. Withdraw the needle and apply gentle pressure with sterile gauze [2].
 - 4.6.1. Talent injecting solution into tail vein.
 - 4.6.2. Close of needle being withdrawn and gauze being applied to the injection site. Videographer's NOTE: 4.5.1, 4.5.2, 4.6.1 and 4.6.2 are combined in one shot.
- 4.7. Return the mouse to its cage on a heating pad [1]. Monitor the mouse for 5 to 10 minutes for signs of distress and record any adverse events [2].
 - 4.7.1. Talent placing mouse back into cage on a heating pad.

- 4.7.2. Shot of mouse being inside the cage. Videographer's NOTE: 4.7.1 and 4.7.2 are combined in one shot.

5. Blood Glucose Monitoring and Analysis

- 5.1. To monitor blood glucose, 7 days after injection, remove the food from mice while maintaining water access for 2 hours [1].
- 5.1.1. Talent removing food from cage.
- 5.2. Then, restrain the mouse and clean the tail tip with 70 percent ethanol [1].
- 5.2.1. Talent holding mouse and cleaning tail tip with 70% ethanol. Videographer's NOTE: Resuse 4.3.1.
- 5.3. Using a sterile lancet or needle, nick the tail and discard the first drop of blood [1].
- 5.3.1. Close-up of tail being nicked and first drop of blood being removed.
- 5.4. Collect the second drop on a glucometer test strip [1].
- 5.4.1. Close-up of second drop blood being collected in a test strip. Videographer's NOTE: 5.3.1 and 5.4.1 are combined in one shot.
- 5.5. Record blood glucose values along with mouse identification and date and repeat measurements twice weekly until the study endpoint [1].
- 5.5.1. Talent recording the blood glucose values in a datasheet.
- 5.6. Define diabetes when values are greater than or equal to 250 milligram per deciliter on two consecutive measurements taken at least 24 hours apart [1].
- 5.6.1. Shot of a talent recording blood glucose values on a datasheet, highlighting two consecutive readings ≥ 250 mg/dL.
- 5.7. At the study endpoint, after euthanizing the mice and extracting the pancreas, perform histological evaluation and islet area quantification [1-TXT].
- 5.7.1. ~~Talent viewing the pancreatic tissue under the microscope.~~ SCREEN: 5.7.1-screen-capture.png **TXT: Euthanasia: CO₂ inhalation**
Videographer's NOTE: This was replaced with screenshot.

Results

6. Results

- 6.1. BmΔvjbR::tnaA-treated mice maintained more stable glycemia [1] compared with vehicle-treated controls [2].
 - 6.1.1. LAB MEDIA: Figure 3A. *Video editor: Emphasize the blue line remaining below the dashed 250 mg/dL.*
 - 6.1.2. LAB MEDIA: Figure 3A. *Video editor: Emphasize the black line above the dashed 250 mg/dL.*
- 6.2. BmΔvjbR::tnaA-treated mice showed delayed onset of hyperglycemia compared with vehicle-treated controls [1].
 - 6.2.1. LAB MEDIA: Figure 3A. *Video editor: Circle and emphasize the point on day 30, where the black line and blue line crosses like X just below the 250 mg/dL (point where black line sharply crosses the 250 mg/gL and blue line remains below 250 mg/dL).*
- 6.3. Quantification of total islet area revealed higher islet area fractions in BmΔvjbR::tnaA-treated mice [1] relative to diabetic controls [2].
 - 6.3.1. LAB MEDIA: Figure 3B. *Video editor: Emphasize the bar labeled TR1 and TL1.*
 - 6.3.2. LAB MEDIA: Figure 3B. *Video editor: Emphasize the bar labeled CL1.*
- 6.4. Diabetic control mice showed severe insulinitis, islet disruption, and reduced insulin staining [1]. Non-diabetic controls and BmΔvjbR::tnaA-treated mice displayed preserved islet architecture and robust insulin expression [2].
 - 6.4.1. LAB MEDIA: Figure 3C. *Video editor: Emphasize the images labelled CL1 in both H&E staining and IHC: Insulin staining.*
 - 6.4.2. LAB MEDIA: Figure 3C. *Video editor: Emphasize the images labelled CR1, TR1, and TL1 in both H&E staining and IHC: Insulin staining.*

Pronunciation guides:

1. Chloramphenicol
 Pronunciation link: <https://www.merriam-webster.com/dictionary/chloramphenicol>
 IPA: /ˌklɔr-ˌam-ˈfen-i-ˌkɒl/
 Phonetic Spelling: klor·am·fen·uh·kaal
2. Tryptic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/tryptic>
 IPA: /ˈtrip-tik/
 Phonetic Spelling: trip·tik

3. Spectrophotometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
IPA: /ˌspek-trō-fə-ˈtā-mə-tər/
Phonetic Spelling: spek·troh·fuh·taa·muh·ter
4. Optical
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>
IPA: /ˈäp-ti-kəl/
Phonetic Spelling: aap·tuh·kuhl
5. Nanometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
IPA: /ˈna-nō-mē-tər/
Phonetic Spelling: nan·oh·mee·ter
6. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsen-trə-ˌfyüj/
Phonetic Spelling: sen·truh·fyooj
7. Endotoxin
Pronunciation link: <https://www.merriam-webster.com/dictionary/endotoxin>
IPA: /ˌen-dō-ˈtāk-sən/
Phonetic Spelling: en·doh·taak·sin
8. Phosphate
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /ˈfäs-ˌfāt/
Phonetic Spelling: faas·fayt
9. Homogeneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>
IPA: /ˌhō-mə-ˈjē-nē-əs/
Phonetic Spelling: hoh·muh·jee·nee·uhs
10. Intravenous
Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>
IPA: /ˌin-trə-ˈvē-nəs/
Phonetic Spelling: in·truh·vee·nuhs
11. Acclimate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimate>
IPA: /ˈa-klə-māt/
Phonetic Spelling: ak·luh·mayt
12. Pathogen-free
Pronunciation link: <https://www.merriam-webster.com/dictionary/pathogen>
IPA: /ˈpa-thə-jən/
Phonetic Spelling: path·uh·juhn
13. Ventilated
Pronunciation link: <https://www.merriam-webster.com/dictionary/ventilated>

IPA: /'ven-tə-ˌlā-təd/

Phonetic Spelling: ven·tuh·lay·tuhd

14. Randomize

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

IPA: /'ran-də-ˌmīz/

Phonetic Spelling: ran·duh·myz

15. Microliters

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

IPA: /'mī-krō-ˌlē-tər/

Phonetic Spelling: my·kroh·lee·ter

16. Ethanol

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

IPA: /'e-thə-ˌnɒl/

Phonetic Spelling: eth·uh·naal

17. Hyperglycemia

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

IPA: /ˌhī-pər-glī-'sē-mē-ə/

Phonetic Spelling: hy·per·gly·see·mee·uh

18. Glucometer

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

IPA: /glu:'kɑ:mətər/

Phonetic Spelling: gloo·kaa·muh·ter

19. Histological

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

IPA: /ˌhis-tə-'lā-ji-kəl/

Phonetic Spelling: his·tuh·laa·juh·kuhl

20. Quantification

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

IPA: /ˌkwän-tə-fə-'kā-shən/

Phonetic Spelling: kwon·tuh·fuh·kay·shuhn

21. Glycemia

Pronunciation link: <https://www.merriam-webster.com/medical/glycemia>

IPA: /glai'si:miə/

Phonetic Spelling: gly·see·mee·uh

22. Insulitis

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

IPA: /ˌɪn-sə-'lai-tɪs/

Phonetic Spelling: in·suh·ly·tuhs

23. Islet

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

IPA: /'ī-lət/

Phonetic Spelling: eye·lut

24. Pancreas

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

IPA: /'paŋ-kre-əs/

Phonetic Spelling: pang·kree·uhs

25. Euthanasia

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

IPA: /,yü-thə-'nā-zhə/

Phonetic Spelling: yoo·thuh·nay·zhuh