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Title: Alternate Immersion in Glucose to Produce Prolonged Hyperglycemia in Zebrafish

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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? **NO**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 11
Number of Shots: 23

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Elizabeth McCarthy:** This protocol induces hyperglycemia in zebrafish, in a rise and fall pattern that mimics the hyperglycemia that is seen in Type II diabetes.

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

1.2. **Elizabeth McCarthy:** The main advantage of this technique is that it is non-invasive and makes it possible to ensure that hyperglycemia is the cause of any altercations observed in the hyperglycemic fish.

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

OPTIONAL:

1.3. **Elizabeth McCarthy:** This protocol could potentially be used to explore therapeutic avenues or pharmaceuticals that target complications of hyperglycemia.

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

1.4. **Elizabeth McCarthy:** This protocol has a lot of steps, but when the steps are followed correctly and with care it should quickly become second nature. Remember however, to always treat the animals gently and humanely throughout the entire protocol.

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

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at American University.

Protocol

2. Preparing the Solution Tanks

2.1. Begin by setting up 6 tanks, 2 for each experimental group. Use 2-liter tanks if the total number of fish is less than 20 and 4-liter tanks if the total number of fish is more than 20. **[1]**. Label one of the two tanks 'housing tank' and the other 'solution tank' **[2]**.

2.1.1. WIDE: Establishing shot of talent in front of the 6 tanks. **TEXT: Experimental Groups: Glucose, Mannitol, and Water**

2.1.2. Talent labeling a tank.

2.2. Keep the tanks in a water bath at 28 to 29 degrees Celsius to maintain water temperature **[1]**.

2.2.1. Talent placing a tank in the water bath.

2.3. On Day 1, place the fish into their respective treatment solutions for 24 hours **[1]**. On Day 2, transfer the fish from their treatment solutions to water for 24 hours **[2]**. On Day 3, transfer the fish from water to treatment solutions **[3]**. *Videographer: This step is important!*

2.3.1. Talent placing fish in a treatment solution, with the tank labeled.

2.3.2. Talent transferring fish in water solution, with the tanks labeled.

2.3.3. Talent transferring fish into the treatment solution, with the tanks labeled.

2.4. Continue this alternating exposure for the remainder of the experiment, transferring water-treated control fish from water to water daily **[1]**.

2.4.1. Talent transferring control fish from one tank to another, with the tanks labeled.

2.5. Ensure that the fish are fed and transferred within the same 2-hour window each day throughout the duration of the experiment **[1]**.

2.5.1. Talent providing food for the fish.

3. Transferring Fish

3.1. Transfer fish in each treatment group from the housing tank to the corresponding solution tank using a standard fish net **[1]**. Place the tank containing the fish back in the water bath **[2]** and replace the airstone and tank lid. This tank is now the 'housing tank' and the tank that previously held the fish is now the 'solution tank' **[3]**. *Videographer: This step is important!*

3.1.1. Talent transferring the fish.

3.1.2. Talent placing the tank with the fish in the water bath.

3.1.3. Talent replacing the airstone and the lid.

3.2. Discard the old solution **[1]** and clean the tank, along with the tank lids, airlines, airstones, and nets to prevent buildup of glucose and mannitol. Use water and a dedicated sponge for each treatment condition to properly clean the tanks **[2-TXT]**.

3.2.1. Talent discarding the solution.

3.2.2. Talent cleaning the tank. **TEXT: Do not wash items with soap**

3.3. Dry the newly cleaned 'solution tanks' with a paper towel and prepare the solutions for the following day using this tank **[1]**. Ensure the other items are dried and separated by appropriate treatment groups **[2]**.

3.3.1. Talent drying a tank.

3.3.2. Properly dried and separated items.

4. Post-transfer Solution Preparation

4.1. To prepare the sugar solutions, fill each solution tank with 2 or 4 liters of System Water **[1]**. Measure the correct amount of glucose and mannitol using a top loading scale and separate weigh boats for each chemical **[2]**. *Videographer: This step is difficult and important!*

4.1.1. Talent filling a tank with system water.

4.1.2. Talent measuring glucose or mannitol, with the glucose and mannitol containers in the shot.

4.2. Add the weighed glucose or mannitol aliquot to the appropriate, cleaned solution tank **[1]**. Stir the solutions with separate glass stir rods until the sugars are completely dissolved **[2]**, then return solution tanks to the water bath **[3]** and cover them with their corresponding lids **[4]**. *Videographer: This step is difficult and important!*

4.2.1. Talent adding glucose or mannitol to the tank. NOTE: This and next shot together

4.2.2. Talent stirring the solution.

4.2.3. Talent returning the tank to the water bath. NOTE: This and next shot together

4.2.4. Talent covering the tank.

4.3. To prepare the water solution, fill experimental tanks with System Water **[1]**. Return these 'solution tanks' to the water bath and cover them with their corresponding lids **[2]**. *Videographer: This step is important!*

4.3.1. Talent filling the experimental tank with System Water.

4.3.2. Talent covering the tank in the water bath.

Results

5. Results: Changes in Blood Glucose, GFAP Levels, and ERG Response in Zebrafish after Induction of Hyperglycemia

5.1. Blood sugar values were significantly elevated after both 4-week and 8-week glucose treatments **[1]**, with hyperglycemia defined as 3 times the control averages from both water-treated and mannitol-treated groups **[2]**.

5.1.1. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the black bars.*

5.1.2. LAB MEDIA: Figure 2 A.

5.2. Retinal tissue collected after 4-weeks of hyperglycemia had an increase in Glial Fibrillary Acidic Protein, or GFAP, levels **[1]**. GFAP expression is observed in Muller glial cells in the retina, which are altered in diabetic retinopathy **[2]**.

5.2.1. LAB MEDIA: Figure 3 A. *Video Editor: Emphasize the black bar.*

5.2.2. LAB MEDIA: Figure 3 A.

5.3. This increase in GFAP was associated with an increase in nuclear factor Kappa B levels **[1]**, suggesting that the induced hyperglycemia triggers an inflammatory response and reactive gliosis **[2]**.

5.3.1. LAB MEDIA: Figure 3 B. *Video Editor: Emphasize the black bar.*

5.3.2. LAB MEDIA: Figure 3 B.

5.4. ERG recordings after 4-weeks of treatment identified a decreased response in glucose-treated retinas compared to mannitol-treated controls **[1]**. Amplitudes of both photoreceptor and bipolar cell components were decreased in hyperglycemic fish **[2]**.

5.4.1. LAB MEDIA: Figure 4 A.

5.4.2. LAB MEDIA: Figure 4 B.

Conclusion

6. Conclusion Interview Statements

6.1. **Elizabeth McCarthy:** This procedure can be supplemented by other tests of hyperglycemia. For instance, a memory assay can be used to look at cognitive deficits, or record visual-based responses such as the optomotor response to assess vision-based complications.

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

6.2. **Elizabeth McCarthy:** Once this technique was established, our lab was able to use it to study hyperglycemia-induced complications in the zebrafish model. We observed these complications relatively quickly – after 4 weeks of treatment.

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