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We use this protocol and it's working

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Saliva Collection

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ABSTRACT

This is a protocol for collection of saliva from mice with pilocarpine stimulation and isoflurane anesthesia.

MATERIALS

Pilocarpine Hydrochloride (Sigma-Aldrich, Cat # 6503)
Sterile isotonic saline
0.2 uM filter
0.6 mL microfuge tubes

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Preparation

1 To prepare a stock solution of pilocarpine hydrochloride (Sigma-Aldrich, catalog # 6503), weigh 10 mg of the compound and dissolve it in 1.776 mL of sterile isotonic saline by vortexing, to give a stock solution of 5.63 mg/ml. There is no need to sterilize this solution. But if desired, filter it through a 0.2 µM filter.

Store this stock solution in 100 ul aliquots in a -80 ^{o}C freezer for up to 3 months. Each frozen stock is for single use. Once thawed, do not refreeze the pilocarpine solution.

2 Take 0.6 mL microfuge tubes, cut of the caps and carefully punch a small hole in the bottom of each tube with a heated 18-gauge needle. Set these tubes into 1.8 mL tubes, which already the caps cut off. The tubes need not be sterile.

3 Using a sharp, sterile razor blade, cut cylindrical absorbent swabs (Salimetrics, catalog # 8048 Swab method, children, 50/pack) into pieces about 2 cm in length. Cut each 2 cm piece diagonally to give 2 conical shaped swabs. Place one swab in each of the 0.6 mL microfuge tubes.

4 Weigh the 0.6 mL microfuge tube containing the dry swab.

5 Keep the mice without food for at least 2 h prior to the start of saliva collection to prevent food particles from contaminating the saliva collected.

6 Just before the end of 2 h, prepare the working pilocarpine solution (0.2815 g/ul) by diluting the stock solution 20x in sterile saline. It is not necessary to further filter sterilize this solution. Always keep the working pilocarpine solution on ice.

Procedure

7 Set up isoflurane unit and attach the “Micky’s space helmet”.

8 Weigh each mouse and determine the dose of pilocarpine for the mouse using **the Excel sheet**. Place the mouse in isoflurane chamber. When the mouse is fully anesthetized, inject the appropriate amount by intraperitoneal route. Set the timer for 2 min and insert the mouse head into the “Micky’s space helmet” at a 45 degree angle, with the head down, and the ventral surface facing upward. Fasten the 2 rubber bands each on both top and bottom teeth to keep the mouth to open.

9 At the end of 2 min, gently insert a swab into the mouth using a pair of dissecting forceps, making sure that the tongue is seen resting on the top of the swab.

10 Grasp the wider end of the swab extending outside of the mouth and rotate it to allow the maximum area of contact with the mouth. Keep the swab in this position for 15 min.

NOTE: This ensures that the swab remains in the mouth for the duration of the collection. Note that the conical tip of the swab acts as a wick and the wider portion of the swab remains outside the mouth.

11 At the end of 15 min, gently rotate the swab to collect any saliva that has not been absorbed and place the wet swab in the 0.6 mL microfuge tube and set it in the 1.8 ml tube placed on ice. Put the mouse back into its cage for recovery from anesthesia.

12 Proceed to the next mouse. All the mice should be monitored until they have completely recovered and are ambulatory.

Measurements

13 At the end of all collections, weigh the 0.6 mL tubes with the wet swab. Calculate the different between the wet weight and the dry weight to get the weight of saliva produced. Place the 0.6 mL tube with saliva back into the 2 mL tube.

14 Centrifuge the 1.8 mL tubes for 5 minutes at 7500xg at 4 degrees C in a microcentrifuge to recover the saliva.

15 Express the results as saliva weight (g) over 15 min or as a ratio saliva weight (g)/mouse body weight (g)