



## ANESTHETIZED CYSTOMETRY

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**Creation Date:** 5/10/2016

**References:**

Bjorling DE, Wang Z-Y, Vezina CM, Ricke WA, Keil KP, Yu W, Guo L, Zeidel ML, Hill WG. Evaluation of voiding assays in mice: Impact of genetic strains and gender. *Am J Physiol Renal Physiol* 2015;308:F1369-1378.

1. Prepare fresh urethane (dissolve urethane in sterile 0.9% saline at 75mg/ml).
2. Cut a piece of PE50 tubing (about 3.8 cm in length). Create flare in one end by dilating the tubing while heating it over a flame.
3. Weigh mouse. Briefly anesthetize mouse with isoflurane (3-5% in oxygen delivered by nose cone or induction chamber).
4. Inject mouse with urethane at a dose of 1.25 – 1.5 g/kg (500ul urethane solution/30g mouse). The response to urethane varies among strains of mice. To avoid overdose, begin at low end of range and adjust. Occasionally, supplemental doses may be needed to maintain surgical plane of anesthesia during testing.
5. Mice are placed on a heating pad or under a lamp to maintain body temperature.
6. Apply lubricant to eyes of mouse.
7. Thirty minutes after injection of urethane, shave lower abdomen of anesthetized mouse. Clean the skin with 70 % alcohol and dry skin with gauze. Incise lower abdomen midline with scissors or scalpel and carefully expose bladder. Use 6-0 silk suture with swaged needle to make a purse string suture near dome. Insert a 25G 1x1/2 inch needle into PE tubing (flared end aligns with the tip of needle). Use needle to puncture bladder wall to introduce the catheter into bladder. Withdraw needle carefully (left the catheter in position), tighten purse string suture, and secure with square knots. Connect the other end of catheter to a blunt 23 gauge needle attached to a 1 ml syringe filled with saline and slowly instill saline into bladder to examine whether catheter is installed properly (bladder should distend, no saline should leak around catheter, and saline should exit urethra).

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8. Close muscle and skin layers separately with 4-0 sutures or wound clips. Cover incision with gauze. Place mouse on heating pad, and allow bladder to recover from acute injury associated with surgical procedure for 1 hour.
9. Flush cystometry apparatus system with saline to remove all air bubbles in the system. Load 15 ml saline to a BD syringe (diameter 21.6 mm, 30 ml size) and place it into the infusion pump that is connected to pressure transducer via a 3-way stopcock. Start infusion pump at a rate of 0.8 ml/hour for 5 minutes before performing the calibration (leave the end of 3-way stopcock that is to be connected to the catheter open).
10. Stop infusion, and close the 3-way stopcock. Connect a sphygmomanometer to the cystometry apparatus. Apply 100 mmHg pressure to the system and record for 1 minute.
11. Infusion pump, pressure transducer, and cystostomy are connected to computer running LabChart version 8 for recording data. Create file for data storage for this experiment.
12. Infuse saline into the bladder at a rate of 0.8 – 1.5 ml/hour to elicit repetitive voidings for about 30 minutes. Continue recording to obtain at least 3-4 consistent voiding cycles. Stop infusion and save file before close the software.
13. The following cystometric parameters are recorded in each animal: baseline pressure (BP; pressure at the beginning of the bladder filling), threshold pressure (TP; bladder pressure immediately prior to micturition), peak micturition pressure (MP), intercontraction interval (ICI; time between micturition events), bladder capacity (BC) that is measured as the amount of saline infused in the bladder to induce micturition, void volume (VV), and nonvoiding bladder contractions (NVCs) that are defined as rhythmic intravesical pressure increases during the filling phase, without the release of fluid from the urethra. Voiding efficiency (VE, %) that is determined as voided volume/bladder capacity x 100.

*End protocol*

## **MATERIALS**

**PE 50 tubing**

**Sterile saline and 70% ethanol**

**6-0 suture with swaged needle**

**Suture or wound clips to close abdominal incision**



**Grass FT03 force transducer for urine balance channels**

**Harvard Apparatus syringe pumps**

**AD Instruments:**

- PowerLab 8/35 (8 channel A-D converter)**
- Bridge Amp (FE224-4 channel or FE228-8 channel)**
- MLT844 pressure transducers**
- MLAC11 adapter cable for Grass force transducers**