Protocol for Removal and Preservation of the Urinary Bladder

Version: 1
Replaced by version: N/A
Edited by: Firuoz Danashgeri , MD

Summary: This protocol describes the proper procedure for harvesting bladders from rats or mice for use by the AMDCC Uropathy group.

Reagents and Materials:

Surgical instruments: skin forceps, 2 micro dissecting forceps, dissecting scissors, scalpel holder w/ size 10 blades or straight blades, holding pins, cutting board, Dissecting microscope or magnifying lens

Reagent/Material	Quantity Required	Vendor	Stock Number
Krebs' solution (has to be made fresh)		See below	
10% neutrol buffered formalin (pH 7.0)			
Lliquid nitrogen and container.			
Cryomold (15×15×15 mm)		Tissue-Tek,	
O.C.T. compound		Tissue-Tek	
2 ml Microcentrifuge Tube (sterilized)		Corning	

Protocol:

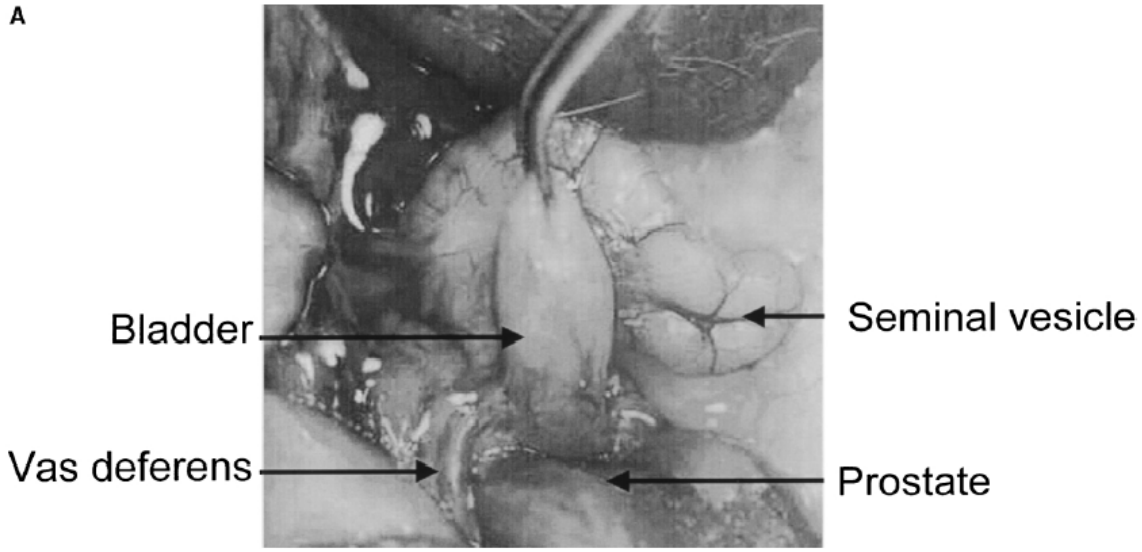
IMPORTANT: *The Kreb buffer must be made fresh each time.. See solution protocol below for recipe.*
Kreb's Solution

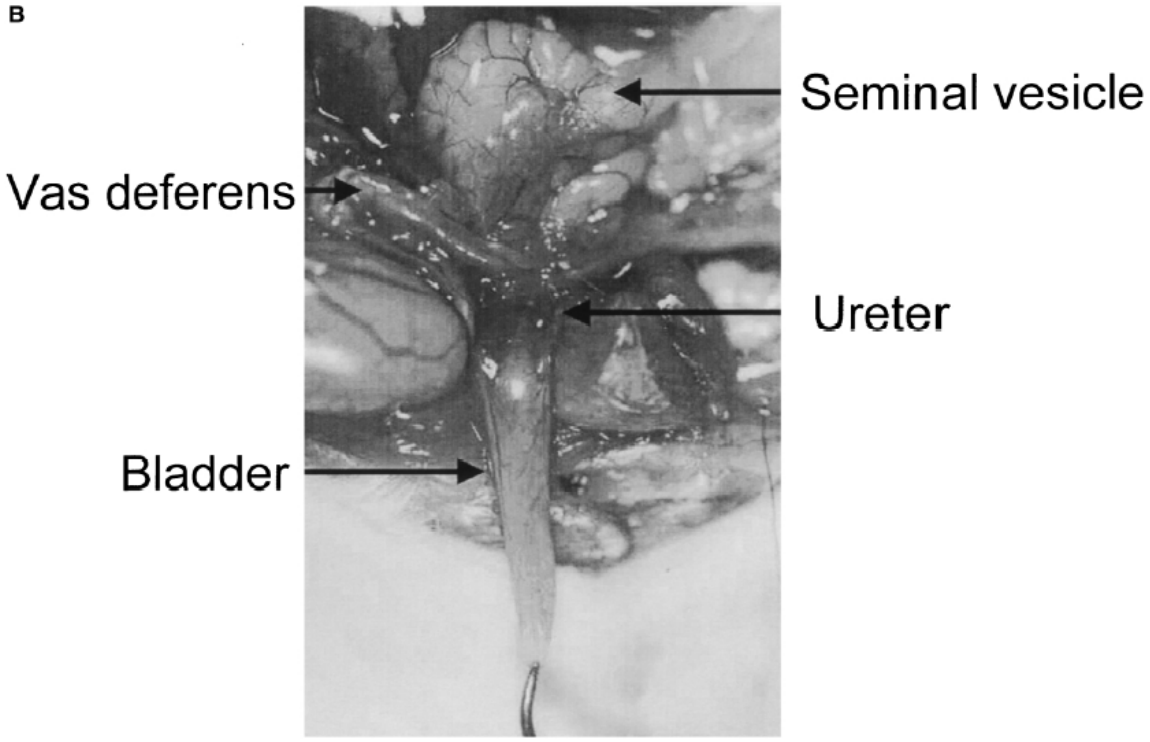
Bladder removal:

1. Anesthetize or euthanize aniamls using approved methods and laid on its back.
2. Make a midline incision in the lower abdominal wall up to the pubic sympheysis.

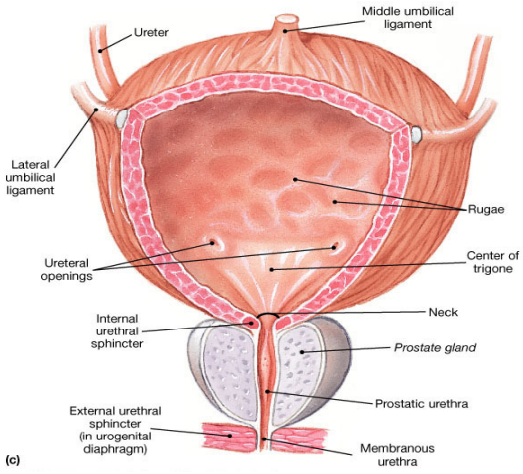
3. Dissect sharply through the muscle layer by lifting up the muscles of the abdominal wall with a pick up on your left hand and making an incision with tips of scissors on your right hand (if you are a right hand). If the incision is complete, you will enter into the abdominal cavity; if it is not repeat incising the muscle layer while you are tenting it up. Tenting up the muscle layer will prevent entering into the abdominal cavity prematurely and injuring intraabdominal organs.
4. The bladder is located in the lower center of the abdomen close to the pelvic bone (see Fig. A). There is a ligament (the median umbilical ligament) connecting the ventral surface of the bladder to the abdominal wall. Cut the ligament.
5. Hold the dome with forceps to gently pull the bladder out of the abdomen in order to dissect it away from other structures (seminal vesicles, vas deferens).
6. Clean the fat surrounding the bladder, prostate, and connective tissue caudally until the urethra is visible as a tubal structure pulling the bladder under the pubic symphesis (Fig. B- urethral is NOT marked). Two other tubal structure attached to the back wall of the bladder are ureters that connect the bladder to the kidneys.
7. Cut the ureters as close as to the bladder.
8. Now the bladder is ready to be removed if all the connective/fat tissue has been separated from
9. Remove the bladder from abdominal cavity by cutting the urethra as far caudally as possible, **weight it** and placed it immediately in physiological buffer (Krebs' solution).

A

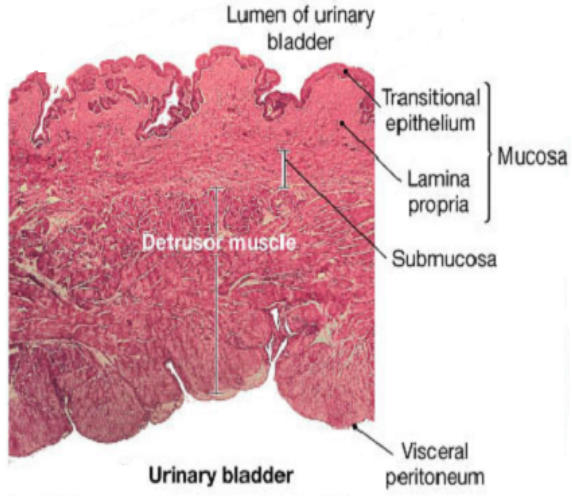



B
Seminal vesicle
Vas deferens
Ureter
Bladder

C


Middle umbilical ligament
Ureter
Lateral umbilical ligament
Rugae
Ureteral openings
Center of trigone
Neck
Internal urethral sphincter
Prostate gland
Prostatic urethra
External urethral sphincter (in urogenital diaphragm)
Membranous urethra
(c)

D


Lumen of urinary bladder
Transitional epithelium
Lamina propria
Mucosa
Detrusor muscle
Submucosa
Visceral peritoneum
Urinary bladder

Bladder Tissue Preparation

Bladder is a globe-shaped hallow organ (Fig. C) made up of two layers of inner mucosal layer (urothelium) and outer muscular layer (detrusor). (Fig. D) When empty it collapses. It is easily picked up by holding into its dome (cephalled) or to the urethra (caudal) by a pair of pick up. For tissue preparation, please follow the below steps:

1. While the bladder is being equilibrated in physiological buffer (Krebs' solution) for at least 10 min, label the containers:
   a. surgical pathology cassettes X 2;
   b. a secure container (centrifuge tube) to hold 40-50 ml Krebs' solution at 4°C
   c. micro centrifuge tubes X2
   d. Crymold
2. Make sure the bladder's weight is recorded. If not, do it NOW and record it.
3. While soaked in Kreb's solution, place the bladder on a hard surface
4. Using a sharp straight blade, cut the bladder into half at its equatorial line. Avoid chain sawing it by using a sharp fresh blade.
5. Using a sharp blade, slice a full circumference section of the bladder from the upper half (cephalled portion) with minimal width of 3 mm.
6. Place the slice obtained from step 4 into a tube containing 40-50 ml Krebs' solution (4°C). This tissue will be used for contractility studies and **should be arrived at our lab within 24 hours.**
7. Place the remaining cephalled portion of the bladder in 4°C phosphate-buffered saline solution for preparation for the next step (Step 13) .
8. Using a sharp blade, slice a full circumference section of the bladder from the lower half (caudal) with minimal width of 3 mm. Place this section in 10% neutral buffered formalin (pH 7.0) in a surgical cassette in room temperature. Make sure the circular orientation of the section of the bladder is preserved in the cassette. This specimen will be used for histological studies.
9. Place the remaining of the lower half (caudal) into the crymold upside down to preserve its anatomical orientation. Use OCT compound to bind tissue to the specimen block and to surround and cover the entire tissue specimen with OCT compound.
10. Snap-freeze the specimen in the OCT compound with liquid nitrogen and stored at –80ºC. This tissue will be used for immunohistochemical studies.
11. *Take a deep breath! And get ready for the next step*.
12. Now you need to separate the urothelium from the muscle of the bladder. The urothelium is loosely attached to the underlying muscle (detrusor).
13. Pin the remaining of the bladder at its four corner to a hard surface. *Use of dissecting microscope or magnifying lens is very helpful for the next step*.
14. Lift up the urothelium with your microdissectig pick up. Push the tips of the microdissecting scissors into the space plane between two layers, while lifting the urothelium.
15. Open the jaws of the scissors while in the plane. This will create an expanded plane between two layers. By repeating this step, you could

separate most of the urothelium from the muscle. Occasionally you need to cut the attachments between two layers.

16. Put urothelium and the muscle into different microcentriguge tubes.
17. Freeze both samples in liquid nitrogen and stored at -80°C. This tissue will be used for Western blotting and RT-PCR
18. Ship the samples from steps 8 (tissue preserved in foramline), 10 (saved in OCT compound) and 17 (snap frozen) to the following address:
    Daneshgari Lab
    The Cleveland Clinic Foundation
    Lerner Research Institute- ND5-08B
    9500 Euclid Avenue
    Cleveland, Ohio 44195
    Tel: 216-445-7886/8196 or 216-444-3677
    Fax: 216-445-0610
    daneshf@ccf.org
    Please fax or email the shipping tracking number to us.

Thank you very much!

Reagent Preparation:

How to Make Kreb's Buffer : **(Hall R, EJP, 2002, 449: 301)**

1. Typical protocol requires approximately 1~3 L.
2. Add following chemicals, fill carboy with appropriate amount of deionized water, stir and gas (95%O_2, 5%CO_2) until dissolved.

CHEMICALS	FW	[mM]	g/1L	g/2L	g/3L
Water	**-**	**-**	**1L**	**2L**	**3L**
NaCl	58.44	133	7.773	15.545	23.319
KCl	74.55	4.7	0.35	0.701	1.051
$NaHCO_3$	84.01	16.3	1.369	2.739	4.107
NaH_2PO_4	119.96	1.35	0.162	0.324	0.486
$MgSO_4 \cdot 7H_2O$	246.48	0.6	0.148	0.296	0.444
Dextrose	180.2	7.8	1.406	2.811	4.218

3. Add following chemicals, stir and gas 10 min

CHEMICALS	FW	[mM]	g/1L	g/2L	g/3L
$CaCl_2 \cdot 2H_2O$	147.0	2.5	0.368	0.735	1.104

4. When not in use, store refrigerated, discard after 24 hours