Video Article

Transurethral Induction of Mouse Urinary Tract Infection

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Abstract

Uropathogenic bacterial strains of interest are grown on agar. Generally, uropathogenic *E. coli* (UPEC) and other strains can be grown overnight on Luria-Bertani (LB) agar at 37°C in ambient air. UPEC strains grow as yellowish-white translucent colonies on LB agar. Following confirmation of appropriate colony morphology, single colonies are then picked to be cultured in broth. LB broth can be used for most uropathogenic bacterial strains. Two serial, overnight LB broth cultures can be employed to enhance expression of type I pili, a well-defined virulence factor for uropathogenic bacteria. Broth cultures are diluted to the desired concentration in phosphate buffered saline (PBS). Eight to 12 week old female mice are placed under isoflurane anesthesia and transurethrally inoculated with bacteria using polyethylene tubing-covered 30 gauge syringes. Typical inocula, which must be empirically determined for each bacterial/mouse strain combination, are 10^6 to 10^8 cfu per mouse in 10 to 50 microliters of PBS. After the desired infection period (one day to several weeks), urine samples and the bladder and both kidneys are harvested. Each organ is minced, placed in PBS, and homogenized in a Blue Bullet homogenizer. Urine and tissue homogenates are serially diluted in PBS and cultured on appropriate agar. The following day, colony forming units are counted.

Video Link

The video component of this article can be found at https://www.jove.com/video/2070/

Protocol

I. Preparation of Urethral Catheters

1. For each mouse bladder and kidney to be sampled, place 5-6 stainless steel beads (bladder, 1.6 mm size or 0.9-2.0 mm blend) or zirconium oxide beads (kidney, 0.5 mm size) in one cryovial tube, respectively. Autoclave the bead-containing tubes.
2. After the tubes cool to ambient temperature, add 200 microliters of sterile PBS to each stainless steel bead-containing tube and 400 microliters of sterile PBS to each zirconium oxide bead-containing tube.
3. We prepare urethral catheters as described by Hung and Hultgren[13]. Autoclave a clean pair of flat-head forceps and fine scissors (iris or similar type) and allow the instruments to cool to ambient temperature. Wipe down the surfaces of a laminar flow hood with 70% ethanol. In the hood, cut a 30 cm (approximate) segment of polyethylene tubing. Aseptically place a sterile 30 gauge needle (1/2 inch long needle) on a sterile 3 cc syringe. Pick up one end of the cut polyethylene tubing with the autoclaved forceps and slide the tubing onto the needle until it meets the hub. Cut the tubing so that approximately 2 cm extends beyond the tip of the needle. Remove the catheter from the syringe and place in a sterile petri dish.
4. To reduce the likelihood of cross-contamination, we make one catheter for each mouse. After all catheters are made, sterilize them by exposure in an uncovered petri dish to UV irradiation for at least 30 minutes. Do not look directly at UV lamps or expose any part of the body in the hood while lamps are activated. After UV exposure, catheters can be stored long-term in the petri dish. We recommend sealing the dish with Parafilm to help maintain sterility.

II. Animal Inoculation

1. Mice should arrive at least a week before experimental manipulation in order to avoid stress-induced confounding factors. Female mice are used because the male mouse urethra is extremely difficult to catheterize. CBA/J is a commonly utilized, UTI-susceptible inbred mouse strain. Mice should be used between 8 and 12 weeks of age, since this is the window of immunological maturity prior to senescence. On the first day of the experiment, dip the end of a sterile inoculating loop into a vial of frozen bacterial stock. Scrape up a small inoculum and streak out on an appropriate agar plate. Generally, uropathogenic *E. coli* (UPEC) and other strains can be grown overnight on Luria-Bertani (LB) agar at 37°C in ambient air. UPEC strains grow as solid, yellowish-white translucent colonies on LB agar. Immediately return the bacterial stock to the freezer. Uropathogenic bacterial strains can become less virulent over time with repeated, serial cultures. Hence, we recommend streaking out a fresh agar plate for each experiment.

2. On day 2 of the experiment, after confirming proper colony morphology, pick single colonies from agar plates and place in at least 5 cc of broth culture overnight. LB broth can be used for most uropathogenic bacterial strains (37°C, 200 rpm in ambient air). Keep the caps loose on the broth culture tubes to allow aeration.
3. On day 3 of the experiment, take 10% of the first broth culture and culture in broth again overnight (typically 37°C and 200 rpm), to enhance type I pili expression. Serial broth cultures help to optimize bacterial uropathogenicity, since type I pili have been shown to be a critical virulence factor for uropathogens *in vivo*1. Keep the caps loose on the broth culture tubes to allow aeration.
4. On day 4 of the experiment, measure the OD_{600} of the broth culture from day 3. If not already known for the bacterial strain being tested, perform ten-fold serial dilution plating over at least 7 logs (dilutions performed with PBS, cultures performed on LB agar, 37°C incubation overnight) to determine the relationship between OD_{600} and cfu/ml. As a rule of thumb, an OD_{600} of 0.4-0.5 commonly corresponds to 1-2x10^7 cfu per 50 microliters. Once the relationship between OD_{600} and cfu/ml has been determined, one can adjust the broth culture to the OD_{600} corresponding to the desired cfu per mouse in 50 microliters of PBS. Some work has suggested that inoculation of 10 microliters, rather than 50, leads to less reflux into the kidneys6. However, most publications have reported use of 50 microliters per mouse. Generally, the desired cfu ranges between 10 6 and 108 cfu/mouse, but each bacterial/mouse strain combination needs to be empirically tested *in vivo*.
5. To help ensure that inocula are not voided out immediately after instillation, mice should be deprived of water for at least 30 minutes prior to induction of general anesthesia. Another key maneuver is to induce voiding prior to anesthesia induction by scruffing mice and gently pressing the lower abdomen. Two to 2.5% isoflurane is a safe induction dose for 8-12 week old female CBA mice, followed by maintenance anesthesia with 1.8-2% isoflurane.
6. Once anesthesia is achieved, mice are placed in the supine position with their head inserted in a nosecone connected to isoflurane-containing oxygen. The lower abdomen is massaged to evacuate urine from the bladder. If the bladder is relatively empty, this maneuver may not yield urine. The lower abdomen and perineum is soaked with 70% ethanol.
7. A sterile 1 cc syringe without a needle is used to draw up the desired bacterial inoculum. Next, a sterile urethral catheter is aseptically placed on the syringe. The excess tubing is cut off the catheter with a pair of scissors so that only 1-2 millimeters of tubing remain distal to the tip of the needle. Then, the syringe is tapped and the plunger depressed with the syringe upright to remove all bubbles. A dollop of bacteriostatic lubricating jelly is squirted onto a sterile petri dish or the inside of sterile syringe wrapper. The tip of the catheter (with attached syringe) is dipped in the jelly. If multiple bacterial strains are being tested in the same experiment, be careful not to use the same drop of jelly to lubricate catheters containing different strains.
8. Using a stereo microscope (0.8x to 5x zoom range) for magnification, the clitoral hood of the anesthetized mouse is gently grasped in the non-dominant hand with a pair of fine-toothed forceps and raised cephalad to expose the deep pink urethral meatus (Figure 1). The syringe (with attached catheter) is grasped in the dominant hand with a pencil grip and the tip of the catheter is engaged in the urethral meatus at a 45 degree angle. The clitoral hood is stretched along the long axis of the syringe, effectively "loading" the urethra onto the catheter. This maneuver is essential since the mouse urethra is redundant. The catheter should slide easily into the bladder (up to the hub, Figure 2), and can be gently twirled to help navigate the redundant folds of the urethra. Once the catheter is hubbed, the syringe can be lowered until it is parallel with the long axis of the mouse. The non-dominant hand can drop the forceps to help stabilize the position of the catheter and syringe as the dominant hand is used to slowly inject the inoculum (at least 5 seconds). If fluid is seen flowing around the catheter during injection, either the bladder is full or the catheter is in the vagina (just caudal to the urethra). Injection should be immediately stopped and the position of the catheter carefully examined. If the catheter was in the urethra, the given mouse should not be included in the experiment. On the other hand, a catheter misplaced in the vagina can be repositioned and the injection re-attempted. After injection, the catheter and syringe is slowly withdrawn from the mouse.
9. To reduce the likelihood of early post-inoculation voiding, which may evacuate administered bacteria and thereby lead to variable infection levels, some investigators maintain mice under anesthesia for 30 minutes after transurethral inoculation10. After surgery, mice should recover on a clean warming blanket with continuous observation to confirm return of normal breathing patterns and activity. Once animals have fully recovered the ability to crawl, they may be returned to bedding-containing cages.

III. Measuring CFU in Infected Tissue

1. Various mouse UTI publications have reported on culture results from 6 hours to one week or longer post-inoculation. The optimal time points need to be determined empirically for each bacterial/mouse strain combination. Voided urine is obtained for culture by scruffing mouse over a sterile Petri dish. Collected urine should be kept on ice at all times. Multiple attempts to obtain voided urine over many hours may be necessary, since mouse voiding is frequent, low volume (30-100 microliters per void), and stress-dependent.
2. After obtaining voided urine specimens, we sacrifice mice using isoflurane and cervical dislocation. The abdomen is aseptically opened. If a voided urine specimen was not successfully obtained earlier, a small amount of urine can often be aspirated through the bladder wall using a 30 gauge needle and syringe.
3. One or both kidneys are removed, minced into at least 4 pieces (each piece should be less than 50 micrograms) on a sterile petri dish, and placed into zirconium bead/PBS-containing cryovials on ice. The bladder is removed, minced into at least 4 pieces, and placed into stainless steel bead/PBS-containing cryovials on ice. The tissue/bead/PBS-containing cryovials are placed in a Bullet Blender homogenizer and the tissues are homogenized using the "8" speed setting for one minute. It is common for some tissue fragments to remain after homogenization. As long as 50-100 microliters of liquid homogenate can be pipetted, solid fragments are not necessarily a problem. If homogenization time is increased for some samples, it is critical to do so for all tissues in order to maintain consistency of homogenization. We utilize a Bullet Blender homogenizer because it avoids the possibility of cross-contamination, is more rapid than processing individual samples one by one using a standard homogenizer, and does not significantly heat the samples being homogenized.
4. At least two or three ten-fold dilutions of collected urine and tissue homogenates should be made in a microtiter plate. For preliminary experiments it may be prudent to make at least seven ten-fold dilutions. Culture up to 100 microliters of urine, bladder, and/or kidney homogenates on appropriate agar. Since urine specimens may be volume-limited, it may be necessary to bring their volumes to 100 microliters prior to performing ten-fold dilutions. If this is required, the initial dilution (before ten-fold dilution) will need to be factored into final calculations of bacterial yields. We prefer culturing UPEC on eosin-methylene blue or MacConkey agar (overnight incubation at 37°C), since these media are selective for Gram negative organisms. UPEC strains grow on MacConkey agar as centrally umbilicated, red-pink colonies due to their ability to ferment lactose.

5. After overnight culture, count the cfu for each plate. For serially diluted samples, the plate dilution containing 50-100 colonies is considered the most accurate count. "Back" calculate cfu per tissue, per milligram of tissue, or per ml.

IV. Representative Results

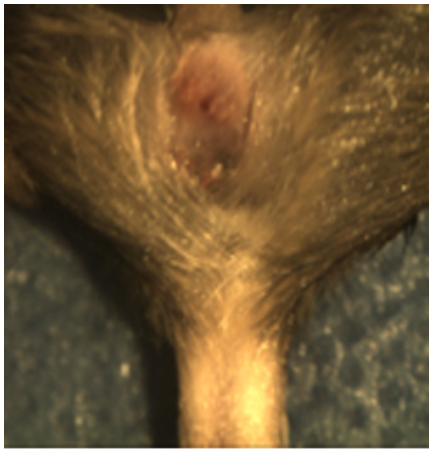

Figure 1. Urethral meatus (dark pink tissue) exposed by lifting clitoral hood cephalad with forceps (beyond top of photo)

Figure 2. Catheterized urethra. The bifid clitoris can be seen just above the catheter.

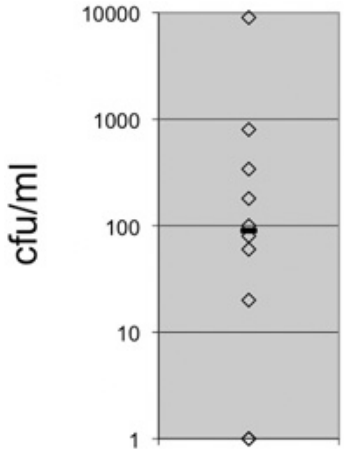

Figure 3. Bladder cfu counts 2 days after transurethral infection of 8 week old female CBA/J mice with uropathogenic *E. coli*. Bladders were homogenized and cfu/ml determined by serial dilution plating on MacConkey agar. Diamonds indicate cfu/ml for individual mice, horizontal bar indicates median cfu/ml.

Discussion

Induction of mouse urinary tract infection by transurethral inoculation of bacteria is a long-established but technically demanding procedure[11]. Hung and Hultgren recently published an excellent review of transurethral techniques of mouse urinary tract infection[13]. In this paper, we attempt to further illustrate the finer points of this experimental approach.

Novices may practice their technique by performing a midline laparotomy to expose the bladder, injecting a dilute solution of India ink (1% in PBS), and observing for blue coloration of the bladder. We have found that a full bladder is one of the most difficult problems to deal with during mouse urethral injection, and is best prevented by the water deprivation and pre-anesthesia voiding strategies outlined above. In our hands, <10% of mice exhibit overflow of urine during urethral injection. Another potential stumbling block is traumatic catheterization, which can lead to urethral perforation, improper doses of bladder inocula, and even animal death. Gentle technique and patience are key during catheterization; successful catheterization should never require forcing the catheter into the bladder.

Finally, some laboratories may not wish to use a Bullet Blender homogenizer or other "medium throughput" homogenizer, either due to low sample numbers or cost considerations. An intermediate solution is to use a standard homogenizer and clean the device between each sample. The most economical solution is use of glass grinders, which we successfully employed prior to switching to the Bullet Blender homogenizer. Regardless of the tissue homogenization method used, meticulous, aseptic, and consistent technique are crucial to obtaining reproducible results.

Disclosures

No conflicts of interest declared.

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