Getting Started with Culex Protocol Writing

Writing protocols for your new Culex equipment can be challenging when you don't yet know the system. We've provided some sample text below to help you get started. Keep in mind that you should use this as a starting point- every institution has different rules about blood draws, caging, experiment timelines, surgical limitations and more. If you need more help with your protocol, let us know; we can't write it for you, but we can help guide you through your questions.

Culex General Description

The Culex Automated Blood Sampling System will be used in this study. The Culex permits the timed, programmed blood collection from catheterized animals. Blood is collected with little or no waste and the animal receives replacement saline solution during each blood draw procedure. Blood is collected in a sterile, closed tubing system and is delivered into a vial that rests in a refrigerated collector. The system can be used for rats, mice and other rodents. With the proper accessories, the system can also facilitate collection of metabolites, bile fluid, tissue dialysates or other physiological measurements. Dosing with Empis and blood collections with Culex can be automated which means that they will occur as programmed without a human in the room. Samples can be collected anytime of the day or night. The software is equipped with a notification system to text (North America only) or email researchers with details of the study. Animals are placed into Culex caging and connected with a light tethering device. Animals remain in these cages for the duration of the study.

Caging Descriptions

Study Type – Rat, No Metabolic Collections

During the experiment, rats will be individually housed in Culex solid bottom plastic cages with bedding. Rats will be lightly tethered while housed in the Culex unit which features a movement-responsive caging system with full range of motion. Appropriate enrichment will be provided.

OR

Study Type – Rat, Metabolic Collections

During the experiment, the animals will be individually housed in Culex metabolic cages with soft wire grid floors. A Teflon coated funnel rests beneath the grid floor to encourage the passive collection of urine and feces without sticking. Feces are separated from the urine with a secondary wire cone inserted in a glass funnel. The glass funnel directs the flow or urine into a vial that rests in a chilling chamber. Urine and feces are collected in a refrigerated container for up to 7 days post-dose. Rats will be lightly tethered while housed in the Culex unit which features a movement-responsive caging system with full range of motion. Appropriate enrichment which does not interfere with the urine and feces collection will be provided.

OR

Study Type – Mouse, No Metabolic Collections

During the experiment, the mice will be individually housed in Culex Universal Cages with a Mouse Floor inserted at the upper fixture. The mouse floor will be covered with bedding. This cage type does not include a food hopper for mice. Food will be given on the cage of the floor. Mice will be lightly tethered using a mouse tether which is shorter and lighter than the rat tether. while housed in the Culex unit which features a movement-responsive caging system with full range of motion. Appropriate enrichment will be provided that does not interfere with the tether or catheter connections on the animal.

Universal and Stackable Cage dimensions: Height: 13 in (33 cm), Diameter: 10.75 in (27 cm)

Tethering

Animals are connected to the balanced tether arm by means of a light collar worn around the neck with an optional shrink wrap or vet wrap cushion for added comfort. Alternatively, a harness, jacket, button or head-mount with a wire connector can be used for connection to the tether. Following surgical recovery in normal housing, rats are placed into the Raturn caging and connected to a balanced tether arm. A light collar (placed around the neck, across the body or between across the neck and one of the front limbs), a harness or a catheter button will be used to connect the animal to the tether.

The tether has a weighted balance that will be adjusted for the animal and cage floor. It is attached to the collar via a spring which depresses a clip, or a magnet if using a button and tether. When the spring is released, the connection is secure. The tubing will be secured to the tether arm using lab tape, there is no incorporated protective coil. With the button and corresponding spring tether, the tubing will be housed within a protective coil.

Movement Responsive Caging

When attached to the collar and tether arm, the animal activates a rotation of the caging to prevent twisting and occlusion of catheter lines. The cage activates only when the animal is moving, and only when the animal has moved past 270 degrees in either direction. Tubing is threaded up through the tether mechanism and out of the cage, where it is connected to external equipment.

This system is an alternative to a liquid swivel. In comparison to a swivel, it increases the number of lines that can be connected without adding torque. It also permits direct connection between the animal and the instrument without additional tubing connections or concentric fluid channels.

The aim of utilizing the movement responsive system is to reduce stress by allowing rats freedom to move about in their cages. However, it is a tethering device and is presumed to add stress relative to complete freedom of movement. Since this system eliminates manual restraint and needle sticks for blood collection, it can be viewed as a refinement to the collection process.

Preparing the Catheter Prior to Connection

Using a corresponding luer stub adapter and syringe, old lock solution in the catheter/ button (approximately 0.05 ml) is aspirated into the syringe and discarded. A new syringe and adapter containing sterile saline or heparinized saline is attached to the catheter; the catheter is flushed with approximately 0.2 to 0.5 ml. The syringe and adapter are disengaged; if using a traditional catheter, the stainless steel pin is replaced in the end of the catheter and, if using a button, the septum automatically closes so no further action is required. If the animal will not be connected to the sample collection tubing within 5 minutes, the locking solution will be replaced in the catheter following the saline flush, and the above steps will be repeated prior to connection.

Acclimation

The animal will be acclimated to the movement responsive system for a period of at least 2 hours and up to 24 hours. For studies that require low stress, acclimation for 24+ hours is recommended. The longer acclimation period allows the animal to go through the active overnight period and return to baseline levels of stress hormones and activity.

For minor surgeries, the animals can be acclimated to the cage during their surgical recovery period.

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Description of Catheter Patency Management While Connected

When connecting to the movement responsive caging, the animal is simultaneously connected to the tubing set of the automated blood sampling system. The plug is removed from the vascular catheter to be used for blood collection and the catheter is connected to the saline-primed tubing line leading down the tether system into the cage. If needed, air bubbles introduced during the connection process can be removed from the connected line through the use of front panel buttons on the control module.

After connection is established, the “TEND” button is depressed to enable the automated catheter flushing program. While tend is enabled, heparinized saline is injected into the catheter line to the animal. The manufacturer recommends using 10 Units/mL of heparin in saline, although concentrations up to 20u/mL are possible. For rats, the system delivers 20 µL of the heparinized saline every 12 minutes while Tend is in use. For mice, the system delivers 10 µL every 12 minutes. During the sampling protocol, Tend is automatically enabled and will occur every 12 minutes unless a blood sample is being collected.

It is essential to use an anticoagulant in the saline solution to ensure proper functioning of the equipment. If the heparinized saline is used for longer than 1 week, heparin should be added to the bag again ito create 10 Units/mL concentration of heparin for the remaining saline. Take care to use the score marks on the bag to determine how much saline remains; do not add the full concentration of heparin as if the saline bag was full.

Description Blood Sampling Procedure

When connected to the system, the catheter is joined with blood collection tubing which is placed in mechanical valves. These valves remain closed unless the front panel buttons are engaged or a blood sampling protocol is in process.

Blood sampling is programmed via software. The user selects both the volume of blood per sample as well as the times that blood is collected. The total amount of blood collected is the responsibility of the programmer, and will adhere to institutional standards of {insert your guidelines here}. The program includes a tally of the total collection volume to assist with this process.

The sampling program is instigated by pressing a button on the machine or in the software. When a sample is collection, the catheter valve opens while a syringe draws blood up the catheter line. More blood is temporarily removed from the animal than is needed for the sample. This is to ensure that the blood delivered to the sample is not mixed/diluted with the saline in the tubing line. The amount of blood that is temporarily removed depends on the total volume of the connected catheter and the collection type. When enough blood has been withdrawn, the catheter valve closes and the collector valve opens. The blood is pushed to the end of a delivery needle, moved to a collection vial and delivered into the vial where it mixes with vial additives. Following sample delivery, all remaining blood is returned to the animal along with a volume of saline that is equal to the amount of blood removed. For a “whole blood LW” (undiluted) blood collection method, there is a loss of 10uL of blood at each time point. This amount is smaller than that lost in a standard needle hub. This amount is accounted for in the total collection volume calculation listed in the software. This process is repeated for every programmed blood sample. At the completion of the blood sampling schedule, the system returns to a standard Tend program to keep the catheter patent.

Description of Dosing Procedure

Catheters to be utilized for dosing are connected to tubing lines that pass up through the tethering system to the top or out of the cage. The catheters are either flushed and/or dosed through these tubing lines using a syringe or an automated programmable infusion pump. They may remain plugged until flushing/dosing or connected to an infusion pump to continuously flush and keep the catheter open.

If manual dosing is required, the movement responsive caging system will be temporarily disabled. Dosing is performed inside of the cage while the animal is still attached via catheter and collar; this may be performed by two technicians. For gavage dosing, the tether may be temporarily detached from the collar to ease passage of the needle. Handling and disconnecting of the animal will be minimized throughout the study to reduce the risk of catheter loss.

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