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Scriptwriter Name: Bridget Colvin
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Title: Use of an Integrated Low-Flow Anesthetic Vaporizer, Ventilator, and Physiological Monitoring System for Rodents

Authors and Affiliations: Krista Bigiarelli[1], Luke E. Schepers[2], Arvin H. Soepriatna[2], Dave FitzMiller[3], and Craig J. Goergen[2]

[1]Pre-Clinical Research and Development, Kent Scientific Corporation
[2]Weldon School of Biomedical Engineering, Purdue University
[3]Marketing Research and Development, Kent Scientific Corporation

Corresponding Author:
Krista Bigiarelli
kbigiarelli@kentscientific.com

Co-authors:
lscheper@purdue.edu
asoepria@purdue.edu
dfitzmiller@kentscientific.com
cgoergen@purdue.edu

Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

First Draft Protocol Length
Number of Shots: **56**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Craig J. Goergen**: This protocol uses best practices for an all-inclusive anesthesia and physiological monitoring suite, including a low-flow electronic vaporizer, integrated ventilator, pulse oximeter, and far infrared warming pad **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Craig J. Goergen**: This methodology is useful for any facility with limited bench or lab space. An all-in-one system also eliminates the need for compressed gas tanks and separate physiological monitoring equipment **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Craig J. Goergen**: Demonstrating the procedure will be Luke Schepers, a biomedical engineering PhD student at Purdue University **[1][2]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Purdue University.

Protocol

2. Low-Flow Vaporizer Hardware Setup

2.1. To set up the low-flow vaporizer, select a carrier gas source **[1]** and remove the red cap from the inlet port on the back of the system to allow the system to take in room air **[2]**.

2.1.1. WIDE: Talent selecting Air Source setting as “Room Air”

2.1.2. Talent removing cap

2.2. If using a compressed gas source, use a pressure regulator and pressure reducer set to 15 pounds per square inch **[1]** and connect the device to the compressed gas port on the back of the system **[2]**.

2.2.1. Talent selecting Air Source setting as “Compressed Gas”
2.2.2. Talent connecting device to port

2.3. Connect the charcoal canister to the exhaust port **[1]** and connect the accessory connector to the inspiratory and expiratory ports on the front of the system **[2]**.

2.3.1. Talent connecting canister to port
2.3.2. Talent connecting connector to port(s) Author NOTE: Ed and I overlooked this step and so we added another shot later on and called it 2.5.2, please refer to this shot number and put it here

2.4. Connect the induction chamber to the branches with blue clips **[1]** and the nose cone to the branches with white clips **[2]**.

2.4.1. Blue clips being applied
2.4.2. White clips being applied

2.5. To set up the integrated ventilator, connect the intubation connector tubing to the branches with the yellow clips **[1]**.

2.5.1. Yellow clips being applied

2.6. To calibrate the ventilator deadspace, on the **Vent Run** screen, touch **Setup** and **Calibration and Tests**. Then select **Deadspace Calibration** and press **Dial B [1]**.

2.6.1. Shot of vent run screen, then setup and Calib & test being touched, Calibration being selected, then Dial B being pressed *Videographer: Important step*
Author NOTE: all yellow highlighted sections were reshot when we realized the screen was running an older firmware version. We updated later on and reshot these portions

2.7. To set up the integrated pulse oximeter, connect the sensor to the MouseSTAT port on the back of the system **[1]**.

2.7.1. Sensor being connected

2.8. To set up the far infrared warming pad, connect the warming pad to the Pad Power port on the front of the system **[1]**.

2.8.1. Talent connecting warm part to pad power port

2.9. Then connect one sensor to the Body Sensor port and the other to the Pad Sensor port and secure the Pad Sensor to the warming pad **[2]**.

2.9.1. Talent connecting sensor(s) to body and pad sensor ports
2.9.2. Talent connecting pad sensor to warming pad

3. System Configuration

3.1. To configure the system settings for anesthesia delivery, in the **Anest Run Screen [1]**, touch **Setup** and select the anesthetic agent **[2]**.

3.1.1. WIDE: Talent selecting screen, with monitor visible in frame
3.1.2. Setup being selected

3.2. Touch **Type Anest** and turn Dial B to select **Isoflurane** or **Sevoflurane [1]**.

3.2.1. Type Anest being touched, and dial being turned/Isoflurane being selected

3.3. To set the syringe size, touch **Syringe Size** and turn dial **B** to select a size **[1]**.

3.3.1. Syringe size being touched, then size being selected

3.4. Touch **Back** to return to the **Anest Run** Screen **[1]** and use the bottle top adapter to fill the syringe with anesthetic **[2]**.

3.4.1. Back being touched

3.4.2. Syringe being filled

3.5. Connect the syringe to the anesthesia system **[1]** and touch **Remove** to move the pusher block backwards as necessary **[2]**.

3.5.1. Talent connecting syringe
3.5.2. Pusher block retracting

3.6. To prime the syringe, press and hold **Prime** to move the pusher block forward until the block just touches the top of the syringe plunger **[1-TXT]**.

3.6.1. Prime being pressed and held/pusher block moving forward *Videographer: Important/difficult step* **TEXT: Do not overprime**

3.7. To set up the mechanical ventilation, open the **Vent Run** screen tab and press **Setup**. Press **Body Weight** and enter the weight of the animal **[1]**.

3.7.1. Vent Run tab being opened, Setup being pressed, Body weight being pressed, then animal weight being entered *Videographer: Important step*

3.8. Then press **Priority** to select "volume" or "pressure-controlled ventilation". The appropriate respiratory rate and tidal volumes will be automatically set **[1]**.

3.8.1. Priority being pressed, then volume or pressure-controlled ventilation being selected, then shot of appropriate rate and volumes

3.9. To set the pulse oximetry parameters, open the **Oxi Run** screen tab and press **Setup**. Press **HR** and turn dial **B** to set the minimum allowed heart rate reading **[1-TXT]**.

3.9.1. Oxi run tab being opened, Setup being pressed, HR being pressed, then HR being set **TEXT: Presets available**

3.10. To set the warming parameters, in the **Warm Run** screen, press **Setup** and select a warming method and a target temperature setting **[1]**.

3.10.1. Warm Run screen being selected, then Setup, warming method, and target temperature being set

4. Anesthesia Delivery

4.1. To anesthetize the mouse, from the **Anest Run** Screen, touch **Start Induction** to begin the airflow **[1]**. The default Induction flow rate is 500 milliliters/minute. Turn dial **A** to adjust the flow rate as necessary **[2]**.

4.1.1. WIDE: Talent pressing start induction
4.1.2. Shot of default flow rate, then dial being turned/flow rate being adjusted

4.2. Place the mouse in the induction chamber **[1]** and close the lid tightly **[2]**.

4.2.1. Talent placing mouse into induction chamber
4.2.2. Talent closing lid

4.3. Adjust the **Anesthetic Agent Concentration** dial to 3% for isoflurane **[1]** and monitor the mouse until it has reached the desired anesthetic plane, as determined by a decrease in respiration rate **[2]** and a loss of righting reflex when the chamber is tipped **[3-TXT]**.

4.3.1. Talent adjusting dial
4.3.2. Shot of mouse rib cage to show respiration rate
4.3.3. Talent tipping chamber *Videographer: More Talent than mouse in shot* **TEXT: Adjust anesthetic agent concentration dial as necessary**

4.4. Once the animal is sufficiently anesthetized, touch **Stop Induction** and **Flush Chamber** to reduce residual anesthetic gas in the chamber **[1]**.

4.4.1. Stop induction and flush chamber being touched

4.5. Open the clips leading to the nose cone **[1]** and close the clips leading to the chamber **[2]**.

4.5.1. Nose cone clamps being opened
4.5.2. Chamber clamps being closed

4.6. Touch **Start Nose Cone** to begin the airflow **[1-TXT]** and immediately fit the nose cone onto the animal **[2]** and center the animal on the infrared warming pad **[3]**.

4.6.1. Start nose cone being touched **TEXT: Body weight setting determines cone flow rate, adjust with dial A**
4.6.2. Cone being fit onto nose *Videographer: Only cone and nose in frame*
4.6.3. Talent centering animal *Videographer: More Talent than mouse in shot*

4.7. Then insert the animal sensor as a rectal probe to control the body temperature **[1]**.

4.7.1. Sensor being inserted

5. Mechanical Ventilation and Physiological Monitoring

5.1. To initiate the mechanical ventilation, place the anesthetized animal on the intubation stage **[1]** and use a thread fixed to the vertical intubation stage to suspend the animal from its upper incisors **[2]**.

5.1.1. WIDE: Talent placing mouse onto stage *Videographer: More Talent than mouse in shot*
5.1.2. Teeth being suspended from thread *Videographer: Important step*

5.2. Gently displace the animal's tongue to the side **[1]** and use the lights provided in the intubation kit to visualize the trachea **[2]**.

5.2.1. Tongue being displaced *Videographer: Difficult step*
5.2.2. Trachea being visualized/lights being shone down throat Author NOTE: We were unable to get a video of the trachea being visualized, there is some footage where we have tried to record this but unsure if it is usable *Videographer: Difficult step*

5.3. Carefully insert the endotracheal tube ~~**[1]**~~. Correct placement can be verified by connecting a small air bladder to the tube ~~**[2]**~~ and checking for lung inflation **[1]**.

5.3.1. Tube being inserted *Videographer: Important step*

5.4. Touch **Stop Nose Cone** to stop the airflow **[1]**. Close the clips to the Nose Cone **[2]** and open the clips to the ventilation tubing **[3]**.

5.4.1. Talent touching Stop Nose Cone *Videographer: Important step*
5.4.2. Nose cone clips being closed *Videographer: Important step*
5.4.3. Ventilation clips being opened *Videographer: Important step*

5.5. Connect the endotracheal tube to the ventilation tubing **[1]** and touch **Start Ventilator** to begin the ventilation **[2]**.

5.5.1. Tube being connected to tubing
5.5.2. Start Ventilator being pressed *Videographer: Difficult step*

5.6. To begin the physiological monitoring, lightly clip the sensor onto one hind paw of the animal **[1]**. The Pulse Oximeter will automatically begin reading the heart rate and the blood oxygen saturation **[2]**.

5.6.1. Sensor being clipped to paw
5.6.2. Shot of oximeter reading HR and SPO2

5.7. Touch the **Oxi Run Screen** tab to view the pulse oximetry data **[1]**.

5.7.1. Oxi Run Screen tab being touched

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.6., 3.6., 3.7., 5.1., 5.3., 5.4.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.6 (Priming) is one of the most critical steps to ensure proper functionality of the integrated vaporizer. Stopping the pusher block just as it touches the top of the syringe plunger is important.
5.2. (Mechanical Ventilation) is also very important. The animal must be connected prior to touching Start Ventilator.

Results

6. Results: Representative Heart Rate and Blood Oxygen Saturation (SPO_2) Analyses

6.1. The heart rate and the blood oxygen saturation **[1]** can be monitored during maintenance via pulse oximetry **[2]**.

6.1.1. LAB MEDIA: Figures 6 and 7 *Video Editor: please emphasize Figure 6*
6.1.2. LAB MEDIA: Figures 6 and 7 *Video Editor: please emphasize Figure 7*

6.2. In this representative analysis, the animals' heart rates and blood oxygen saturation remained stable, with few significant changes observed in either measurement for all of the groups **[1]** and with the blood oxygen saturation remaining between 82-99% **[2]**.

6.2.1. LAB MEDIA: Figures 6 and 7
6.2.2. LAB MEDIA: Figures 6 and 7 *Video Editor: please add dotted horizontal lines at 82 and 99 percent in all Figure 7 graphs*

Conclusion

7. Conclusion Interview Statements

7.1. **Luke Schepers**: This technique allows researchers to deliver anesthesia at low flow rates without the need for compressed gas. Lower flow rates lead to less gas waste and less exposure for research personnel **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera