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# A SIMPLE METHOD OF ENDOTRACHEAL INTUBATION IN MICE

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*Abstract* - Endotracheal intubation in mice is commonly used when airway management is necessary. The procedure is difficult because of the small size of the animal and its oropharynx. Various methods for endotracheally intubating mice have been described in the scientific literature, and most of these methods require the use of expensive devices and/or special training. In this report, we describe our experience of an easy, reliable, and inexpensive method for endotracheally intubating mice through the mouth, developed in our Center, using low-cost materials, which can be found in almost all surgical laboratories.

*Key words:* Mouse, endotracheal intubation

## INTRODUCTION

Endotracheal intubation is done for (a) airway management and the mechanical ventilation when animals are anaesthetized with an inhalation anesthetic agent, (b) the intratracheal instillation of a test compound, or (c) assessment of pulmonary function. Depending on the specific needs of the research, the endotracheal tube can be either inserted into the trachea through the mouth (orotracheal intubation) or by performing a tracheostomy (Spoelstra et al., 2007; Miller et al., 1999; Hoetzel et al., 2009; Huang et al., 2010). Of the two methods, orotracheal intubation is less traumatic, and can be repeated in the same animal (Spoelstra et al., 2007).

The endotracheal intubation of mice is extensively described in the scientific literature, and these published methods vary in technique and the required equipment (Spoelstra et al., 2007; Cambron et al., 1995; Brown et al., 1999). Endotracheal intubation of mice is difficult to do because of the mouse's small body size and its narrow mouth cavity and glottic opening. This smallness necessitates the use of specific and often expensive equipment, such as a surgical microscope, a flexible or rigid fiber optic laryngoscope, an otoscope or other custom-made instruments, to endotracheally intubate a mouse (Spoelstra et al., 2007; Costa et al., 1986; Hastings et al., 1999; Hamacher et al., 2008).

## DESCRIPTION OF THE METHOD

We have developed a non-traumatic method for rapid and repeated endotracheal intubation of mice through the mouth (orotracheal intubation). In this method, the following materials are required: a 22G Teflon intravenous cannula, one disposable insulin syringe, one pair of anatomic forceps, a powerful flashlight, 4/0 coated, braided silk, non-absorbable

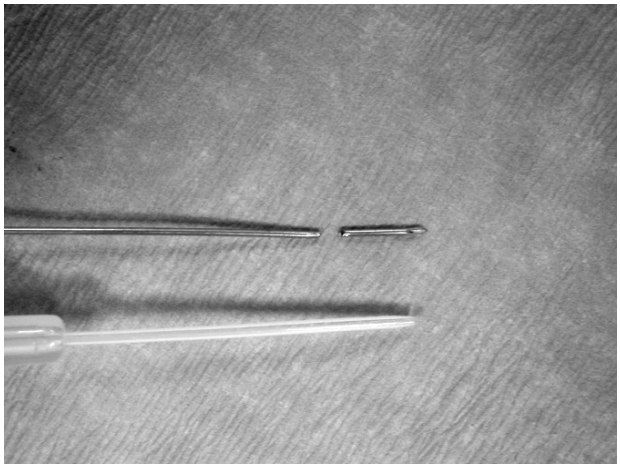

**Fig. 1.** The tip (approximately 5mm) of the metal needle of the Teflon intravenous cannula is cut before passing it into the mouse's trachea.

**Fig. 2.** Immobilization of the mouse forelimbs using surgical tape and the upper incisors using a 4/0 silk suture.

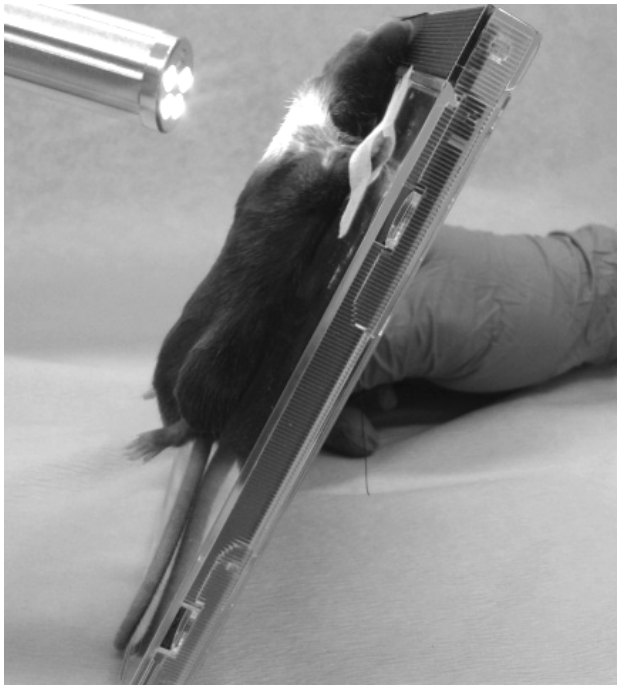

**Fig. 3.** The anaesthetized mouse is secured to a plastic compact disc case, which is then placed in a head-up position at an angle of approximately 60-70°.

surgical suture (Silkam® B/BRAUN Aesculap AG & CO, Tuttligen, Germany), 10% lidocaine (Xylocaine®, Astra-Zeneca, Sodertalje, Sweden), surgical tape, a plastic compact disc (CD) case, which is used as the working table, normal saline solution, gauze, and one pair of scissors. The 22G Teflon intravenous cannula is used as the endotracheal tube. The tip (approximately 5 mm) of the metal needle of the intravenous cannula is cut in order to prevent the needle damaging the tissues while intubating the mouse (Fig. 1). The rest of the needle is used as a guide wire in the catheter in order to keep the tracheal tube straight for intubating the mouse. Before passing the catheter into the trachea, its tip is sprayed with 10% lidocaine in order to facilitate its passage by preventing laryngospasm and numbing the throat during its passage. The mouse is usually anaesthetized using a combination of 85 mg/kg ketamine (Imalgene 1000®, Merial, Lyon, France), and 10 mg/kg xylazine (Rompun®, Bayer Animal Health GmbH, Leverkusen, Germany). Alternatively, the mouse can be anaesthetized using intraperitoneally-injected medetomidine (1mg/kg; Domitor®, Orion Corporation, Turku, Finland) combined with 3.5% isoflurane (Forenium®, Abbott Laboratories Ltd, United Kingdom), which is delivered through a face mask at a flow 0.6 liters/minute. The depth of anesthesia that is provided by this method is sufficient for orotracheal intubation and the effects of medetomidine can be reversed with intraperitoneally injected atipamezole (2mg/kg; Antisedan®, Orion Corporation, Turku, Finland) for rapid recovery from the anesthesia.

**Fig. 4.** The tongue is held with a small piece of gauze and the intravenous cannula is inserted into the mouth.

The depth of anesthesia is determined by the absence of swallowing and righting reflexes. The presence or absence of the righting reflex can be determined by pinching the rear foot or the tail of the mouse. When the righting reflex is abolished, the anaesthetized mouse is placed on the CD case in a supine position. Its forelimbs are immobilized with surgical tape and its upper incisors are secured with the 4/0 silk suture (Fig. 2).

The anaesthetized mouse is then suspended at an angle of 60-70° on its back in a head-up position on the CD case (Fig. 3). The mouse's tongue is gently pulled out of the mouth using anatomical forceps

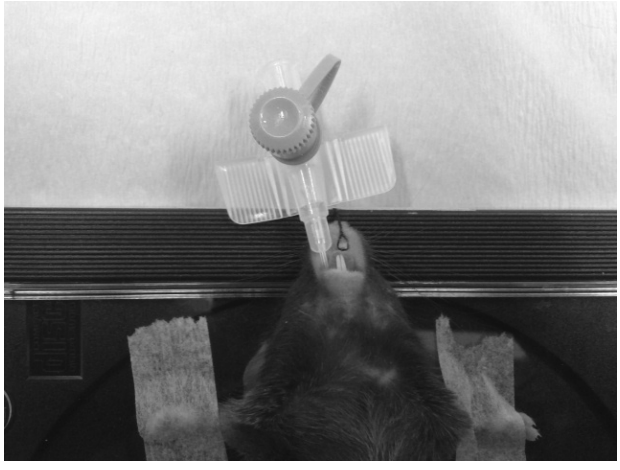

**Fig. 5.** The intubated anaesthetized mouse.

and is held aside using the thumb and index fingers. In order to avoid the tongue slipping from the fingers, a small piece of cotton gauze can be used to improve the grip of the fingers. The area of the vocal cords is transilluminated in order to permit a clear view of the tracheal opening by focusing the light beam of the flashlight, which is held by an assistant, on the ventral neck of the mouse (Fig. 4). The intravenous catheter is inserted into the mouth, and the epiglottis is opened by circular movements of the catheter's tip. The catheter is then advanced through the larynx and across the vocal cords into the trachea. Resistance and laryngospasm may be encountered if the tip of the catheter touches the vocal cords. In such circumstances, the catheter should be retracted and the procedure repeated. When the mouse is intubated, the needle of the catheter is removed carefully and the CD is restored to the horizontal position (Fig. 5). Finally, the tube is secured with surgical tape, and the intubated mouse is ready to be connected to the ventilator.

A small dental mirror can be used to confirm the correct placement of the catheter in the trachea. For this purpose, the mirror should be chilled in a -20°C freezer and kept cold until use, and positioned over the catheter's visible inlet after placing the catheter. If the catheter has been successfully placed in the trachea, then a visible condensate will form on the mir-

ror's surface due to the moist exhaled air. Although this method of confirming the correct placement of the catheter has been described as unreliable because the formed mist is occasionally vague (Watanabe et al., 2009), we found this method reliable for confirming the correct placement of the endotracheal tube. Other published methods for confirming proper placement of the endotracheal tube in mice include closing the end of the tube with the fingertips or placing a plastic pipette onto the end of the tube or an extension tube of intravenous infusion that contains one drop of water (Watanabe et al., 2009; Rivera et al., 2005). In our opinion, all these methods for confirming proper placement of the endotracheal tube can potentially compromise the respiratory function of the mice.

## DISCUSSION

The endotracheal intubation of mice requires special skills. Based on our experience, the described technique is easy to learn and can be adopted even by moderately experienced personnel. Although the method requires an assistant in order to perform the intubation, we never faced any coordination problems in the everyday practice. The main advantage of the method is that it does not necessitate the use of expensive instrumentation such as a surgical microscope, special tables, extra guide wires, or instruments such as a laryngoscope or an otoscope. It is used routinely in our Center in different research protocols that require the monitoring of airway management and the ventilation of anaesthetized mice. To date, we have used this method of orotracheal intubation in more than 200 mice, and all animals have recovered without any problems or residual adverse effects.

## CONCLUSION

Herein we have described an easy, reliable and inexpensive method for endotracheally intubating mice through the mouth using low-cost materials that can be found in almost all surgical laboratories. Investigators and laboratory animal technicians with relatively little experience can be trained to use this method of orotracheal intubation when they need to intubate mice in their research.

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