

# Access to the tracheal pulmonary pathway in small rodents

## *Vias de acesso traqueopulmonar em pequenos roedores*

Roberto S. A. Ribeiro<sup>1</sup>; Isabeliza M. E. S. R. Ferreira<sup>1</sup>; Israel Figueiredo Jr<sup>2</sup>; Maurício A. Verícimo<sup>1</sup>

1. Departamento de Imunobiologia, Instituto de Biologia, Universidade Federal Fluminense (UFF). 2. Departamento Materno-Infantil, Hospital Universitário Antônio Pedro, UFF.

### ABSTRACT

The tracheal pulmonary route is used in diverse experimental models for the study of drugs, infectious agents, and diseases. In view of its importance and associated difficulties, the present article proposes to give research groups up-to-date information on techniques to access the tracheal pulmonary pathway of small rodents.

**Key words:** tracheal pulmonary; small rodents; tracheotomy; tracheostomy; intranasal administration; orotracheal intubation.

### INTRODUCTION

Access to the airways of laboratory animals is important in experimental models to study the action of infectious agents, the instillation of drugs and the practice of mechanical ventilation, among others. Airway access can be obtained by a variety of techniques, with each chosen procedure demonstrating advantages and disadvantages. These techniques are divided into surgical (tracheostomy and tracheotomy) and non-surgical (intranasal instillation and orotracheal intubation) procedures. The surgical techniques are the most commonly used due to their precision; however they are invasive<sup>(1-3)</sup> and associated with various complications, such as airway trauma, swelling/edema and hemorrhage<sup>(1)</sup>. Non-surgical techniques are less used, but non-invasive. They are rapid and easily executed<sup>(3)</sup>, however they present a lesser degree of precision than surgical procedures. When choosing a technique to access the tracheal pulmonary pathway, the researcher should be well informed on the limitations and potential consequences of each procedure. Therefore, the objective of this study is to provide the necessary information to choose a suitable technique for the experimental protocol of the research.

### SURGICAL ROUTE

#### **Tracheotomy × tracheostomy**

In the literature, the terms tracheotomy and tracheostomy are often used interchangeably, but there are differences. The term tracheotomy comes from the Greek *trachea* and *tomia*, which is a variation for *tomo* (incision, division). Thus, tracheotomy may be defined as the surgical opening of the trachea for any medical or research purpose. In tracheostomy, the element *stoma* is added, which refers to the connection of the trachea to the outside through a surgically created opening. This connection is performed by the insertion of a cannula in the tracheal lumen<sup>(4)</sup>. Unfortunately, studies which employ these techniques in laboratory animals commonly use the nomenclature erroneously, describing for example, the tracheostomy technique and naming it as tracheotomy<sup>(5,6)</sup>.

#### **Puncture tracheotomy**

The technique of puncture tracheotomy was previously described for experimental infection<sup>(7-9)</sup>, instillation of drugs<sup>(10)</sup>

and bronchoalveolar lavage<sup>(11)</sup>. To perform the procedure, the animal should be anesthetized and placed in the supine position to extend the cervical region and facilitate the tracheal puncture. This is accomplished through the skin, separating the adjacent subcutaneous tissues and the salivary glands. Removal of hair below the mandible and local asepsis before the incision are indicated. The puncture is generally carried out with a syringe attached to a needle in parallel with the trachea<sup>(12)</sup>. Skin can be sutured<sup>(9)</sup> following the procedure, or a cyanoacrylate adhesive solution can be applied<sup>(13)</sup>. **Figure 1** shows the suitable space for performing tracheotomy.

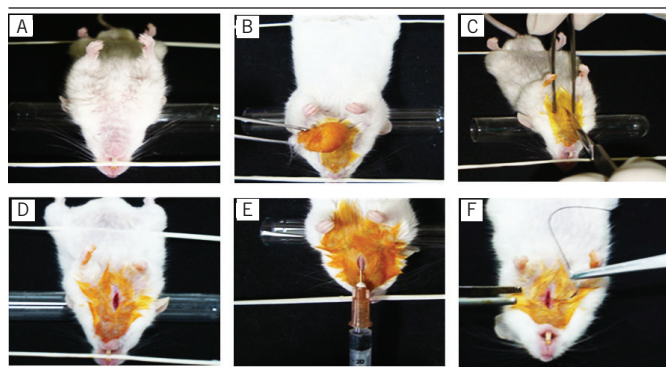


FIGURE 1 – Puncture tracheotomy in BALB/c lineage mice

A) mouse in the supine position; B) asepsis of the cervical region; C and D) incision of the skin; E) puncture and inoculation; F) skin suture.

### Tracheostomy

The use of tracheostomy was previously described in mechanical ventilation models<sup>(14, 15)</sup>, myocardial infarction in small rodents<sup>(16)</sup>, and bronchoalveolar lavage<sup>(17)</sup>. To perform the technique, the animal must be positioned correctly. After anesthesia, animals should be placed in the supine position, with extremities fixed on the operating platform. The neck should be extended to facilitate tracheal incision. Similarly to tracheotomy, hair removal and local asepsis aid the technique. The incision should be made in the midline below the neck. Salivary glands should be separated, and the muscles surrounding the trachea should be fixed by sutures. After viewing the trachea, an incision is made to insert the cannula between the fourth and the fifth tracheal ring<sup>(15)</sup>.

### Non-surgical route

The use of less invasive procedures has been encouraged after the establishment of the three R's (replacement, reduction and refinement) described by Russell and Burch in 1959. Hence, the

substitution of more invasive surgical procedures is valid as an ethical model<sup>(18)</sup>.

Although the surgical techniques are more frequently employed, they are rarely modified: similar protocols are carried out by different research groups. Conversely, there are many methodological research articles on non-surgical routes of tracheal pulmonary access, especially for orotracheal intubation. As a result, the non-surgical techniques possess multiple variations and different protocols, requiring a more detailed description.

### Intranasal instillation

The intranasal route is used for the administration of infectious agents<sup>(19-21)</sup>, drugs<sup>(22, 23)</sup> and the sensitization to antigens<sup>(24)</sup>. Unlike the other techniques mentioned here, this technique allows the instilled volume to travel from the nasal passages to the lungs. To carry out nasal instillation, the animal should be strongly anesthetized; and the volume to be instilled, slowly expelled into the nostrils, generally by the use of a micropipette<sup>(25)</sup>. In agreement with Southam *et al.*<sup>(26)</sup>, the instillation of volumes smaller than 5  $\mu$ l is not sufficient to reach the lungs. The efficacy of instillation increases proportionally to the volume instilled, however volumes greater than 60  $\mu$ l should be divided into two instillations separated by a pause of approximately 10 minutes to increase the efficacy of the technique<sup>(21)</sup>. Body position does not influence instillation; however it should be noted that non-anesthetized animals show a greater incidence of stomach instillation in comparison with anesthetized ones<sup>(26)</sup>.

Morrison *et al.*<sup>(27)</sup> and Barlow *et al.*<sup>(28)</sup> reported the performance of instillation by nebulization, in which the animal is placed in a cabinet with insulation and high-efficiency particulate air (HEPA) filters, and the solution to be instilled is mixed with carrier gas (95% O<sub>2</sub> and 5% CO<sub>2</sub>), according to Morrison<sup>(27)</sup>. Although efficient, the technique requires the use of commercial kits which are costly and therefore often unobtainable for many research groups.

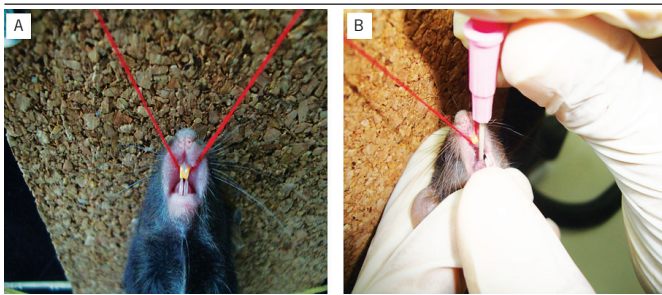
### Orotracheal intubation

Orotracheal intubation in laboratory animals can be carried out by non-surgical means for endotracheal application of drugs, infectious agents or mechanical ventilation<sup>(29-30)</sup>. In order to carry out the technique, the rodent is anesthetized and placed on a horizontal, vertical or inclined support. The trachea is well illuminated, and intubation is performed usually by a small plastic cannula. The use of accessory instruments to facilitate the

intubation process, such as laryngoscope and arthroscope, has been reported.

### Positioning the animal

Visualization of the vocal chords is an important factor in orotracheal intubation of rodents. For this reason, it is necessary to position the animal adequately (alignment of the oropharyngolaryngeal axis). The current literature on this topic gives superficial information on how to achieve the best positioning, and the descriptions are summarized only, making experimental reproducibility difficult. Some studies state that animals should be maintained horizontally<sup>(31)</sup>, vertically<sup>(32)</sup>, or inclined with the aid of a support. Other studies include photos of the supports used, however they do not specify the degree of inclination<sup>(33)</sup>. Studies concisely detailing the positioning of the animal report the suspension of the rodent by a nylon thread through the incisors at an inclination of between 30° and 45°<sup>(30, 34, 35)</sup>. Studies stating that the positioning of the animal should be on a horizontal or vertical surface do not mention the height at which the operator should be in relation to the support<sup>(31, 32)</sup>. Alternatively, Hamacher *et al.*<sup>(36)</sup> described the inclination of the animal through its handling by the operator, facilitating the adjustment of the inclination angle (**Figure 2**).



**FIGURE 2** – Adapted intubation method from Hamacher *et al.*

*A) anesthetized mouse in the vertical position on the platform; B) handling the cannula during the murine intubation process.*

### Light source

Good lighting is essential for viewing the vocal chords during the orotracheal intubation process, avoiding possible esophageal intubation<sup>(37)</sup>. Illumination through the thin/fine skin of the neck region (translumination) by a small halogen/halothane lamp has been widely used by many research groups<sup>(30, 34, 35)</sup>. This technique allows the clear visualization of vocal cord movement with correct positioning of the animal. The use of optical fiber as a light source also aids the instillation process, as reported by MacDonald and

Hamacher<sup>(36, 38)</sup>. In these studies, the optical fiber is coupled inside the cannula used for intubation. The use of an otoscope also has shown to be an effective procedure. Once placed correctly in the oral cavity, the otoscope provides abundant illumination and allows the operator a large image of the trachea<sup>(31, 39)</sup>.

### Accessory equipment

The use of accessory equipment emerged with the objective of improving pre-existing intubation techniques, facilitating the stable positioning of the animal and the visualization of the trachea.

Immobilisation of the rodent's tongue is important during intubation, with the use of tweezers<sup>(35)</sup> having been previously described. However, the use of a laryngoscope is more commonly cited in studies to avoid possible injury to the rodent's tongue<sup>(30, 34, 40, 41)</sup>. The preparation of a homemade laryngoscope is possible for this procedure, achieving similar results to the previously mentioned groups while lowering the cost<sup>(1)</sup>. Use of the operator's fingers to position the tongue was reported by Hamacher and MacDonald<sup>(37, 38)</sup>, however caution should be taken. Unnecessary force should not be used, and the tongue should not be placed on the animal's incisors, what would result in abrasion or perforation of the tongue.

The use of videoendoscopy to replace lighting for viewing the trachea was first reported by Vergari in 2004<sup>(42)</sup>. This technique increases reproducibility and facility of the intubation process through assisting cannula placement in the trachea by a computer in real time. To carry out the videoendoscopy, an arthroscope is coupled with a computer camera, and the cannula is inserted into the oral cavity of the anesthetized animal<sup>(42, 43)</sup>.

To confirm the orotracheal intubation process, the use of computed tomography (CT) imaging has been reported by Buckle *et al.*<sup>(33)</sup>. In this study, bioluminescent lung tumor cells were instilled in the orotrachea of BALB/cABOmA-nu mice and subsequently visualized by single photon emission computer tomography (SPECT).

## DIFFICULTIES ASSOCIATED WITH THE TECHNIQUES

Surgical techniques are traditionally the most used by research groups to access the respiratory pathway of small rodents. They allow the instilled volume to reach the animals' lungs with high accuracy, without loss along the upper respiratory tract. Nevertheless these techniques are considered invasive<sup>(2)</sup>,

often leading to undesirable consequences, depending on the experimental model, due to the inflammatory process and the possible scarring<sup>(44)</sup>. The tracheotomy technique is also considered to induce stress, apathy and anxiety, as demonstrated in C57BL/6<sup>(45)</sup> and BALB/c<sup>(37)</sup> lineage mice, what is an aggravation for experiments associated with behavioral tests. A possible solution to reduce these effects would be to puncture the animal's skin without the need to surgically open the skin; however there are no reports of this technique in the literature, and a morphological study is necessary for its development.

Intranasal instillation may be considered the best choice for experiments that aim to study infection by airborne pathogens or inhaled drugs, since it reproduces the pathway made by the instilled volume from the nasal passages to the lungs. However, instillation of volumes smaller than 5 µl will not effectively reach animals' lungs. It should also be considered that even after instillation of greater volumes, a part remains in the upper respiratory tract due to the high viscosity of the nasal mucosa<sup>(26)</sup>, preventing the reproducibility of the technique and leading to the spread of the inoculum to extrapulmonary tissues<sup>(37)</sup>. Also, special care should be taken in the management of animals receiving nasal instillation of infectious agents or drugs, with the use of personal and collective protection equipment as well as adequate training being essential.

Orotracheal intubation allows virtually the total instilled volume to reach the animals' lungs, without passing through the upper respiratory tract. Although rapid, this procedure requires appropriate instrumentation, which is often difficult to obtain or costly. So far, complications related to the technique have not been described. Nevertheless, our research group believes that certain difficulties presented in humans, such as selective intubation, alveolar rupture, mucosal ulceration, among others, may occur, requiring the operator to have practical training.

## FINAL CONSIDERATIONS

The present study describes the surgical and non-surgical routes for tracheal pulmonary access. Here we do not describe the thoracotomy, which is not a focus of this study, since it allows pulmonary access, but not via the trachea. As reported, the use of non-surgical experimental models has grown in popularity due to their less invasive characteristics, which reduce possible injury and distress to the animal. The choice of a technique for tracheal pulmonary access depends upon the research to be conducted and the structure of the individual laboratory, so that reliable results can be achieved.

## RESUMO

*As vias de acesso traqueopulmonar vêm sendo utilizadas em diversos modelos experimentais que estudam a ação de fármacos e agentes infecciosos, além de enfermidades. Tendo em vista a sua importância e as dificuldades associadas, o presente artigo de atualização propõe-se a dar ao pesquisador as informações necessárias para o emprego das técnicas de acesso traqueopulmonar em pequenos roedores.*

*Unitermos: traqueopulmonar; pequenos roedores; traqueotomia; traqueostomia; administração intranasal; intubação oro-traqueal.*

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#### MAILING ADDRESS

Roberto Stefan de Almeida Ribeiro

Universidade Federal Fluminense; Instituto de Biologia; Departamento de Imunobiologia; Laboratório de Imunologia das Doenças Infecciosas e Granulomas; Campus Universitário do Valonguinho; Alameda Barros Terra, s/n; Centro; CEP: 24020-150; Niterói-RJ, Brazil; e-mail: robertostefanbio@gmail.com.