

## New Visible Endotracheal Intubation Method Using the Endoscope System for Mice Inhalational Anesthesia

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(Received 26 December 2013/Accepted 16 February 2014/Published online in J-STAGE 3 March 2014)

**ABSTRACT.** Appropriate and effective anesthesia is critical, because it has a strong influence on laboratory animals, and its affect greatly impacts the experimental data. Inhalational anesthesia by endotracheal intubation is currently prevailing in general anesthesia and is preferred over injection anesthesia, especially for large laboratory animals, because it is a safe and easy control agent. However, it is not common for small laboratory animals, because of the high degree of technical skills required. We assessed the capability of use for mice of the endotracheal intubation by using the endoscope system “TESALA AE-C1” and inhalational anesthesia using a ventilator. Endotracheal intubation was successfully performed on all 10 C57BL/6 mice injected with M/M/B: 0.3/4/5 comprised of medetomidine, midazolam and butorphanol, at a dose of 0.3 mg/kg + 4.0 mg/kg + 5.0 mg/kg body weight/mouse, respectively. After the intubated mice were connected with the inhalational anesthesia circuit and the ventilator, vital signs were measured until 15 min after the connection. The data with M/M/B: 0.3/4/5 showed stable and normal values, which indicated that this new endotracheal intubation method was simple, reliable and safe, which mean that this anesthesia is favorable in regard to the animal’s welfare.

**KEY WORDS:** 3Rs, anesthesia, endoscope, intubation, mouse.

doi: 10.1292/jvms.13-0647; *J. Vet. Med. Sci.* 76(6): 863–868, 2014

Anesthesia strongly influences the experimental condition of laboratory animals, and it can also greatly affect the experimental data. Therefore, appropriate and effective methods are critical for safe and reliable animal experimentation [6–8, 12, 16, 22]. In animal experiments using laboratory rodents, such as mice and rats, injectable anesthesia is often the general anesthesia, but it is difficult to control the depth, the duration and the time of metabolization. Furthermore, the inhalational anesthesia overcomes the complications of injectable methods and supplies balanced effects, which are composed of sedation, analgesia and muscle relaxation, however, this technique requires complex and expensive equipment [4, 6–8, 16, 22]. Two kinds of inhalational anesthesia methods are generally used for experimental rodents; the face mask method and the endotracheal intubation method. The former is simple, but relies upon the spontaneous breathing of the animal. Because of this, the animals

sometimes die of spontaneous ventilatory arrest with deep anesthesia. In addition, it is unsuitable for open heart surgery and dysfunction of the heart and lungs. The latter is a safer and easier method to control the depth and duration of the anesthesia when compared to the face mask method. The operator can deliver the anesthetic and oxygen to the alveolus using a ventilator to control the breathing rate of the rodent. Hence, ventilatory trouble is less likely to occur due to the muscle relaxation effect of the anesthetic, as well as using the ventilator to control the breathing rate [2, 4, 6–9, 16, 18, 22]. As a result, the inhalation anesthesia by endotracheal intubation method is relatively safe and suitable for animals, especially those with the cardiopulmonary dysfunctions and/or under surgical operation. However, endotracheal intubation is not suitable for mice, because they possess a small body size, and, because the methods of endotracheal intubation of the mice comparatively require skillful techniques and special equipment. Therefore, a suitable system has not yet been published [3, 10, 15, 17, 19, 21]. In articles on the murine endotracheal intubation for mice and rats [3, 10, 15, 19, 21], consideration of the health of the animal is essential. For example, the lack of monitoring of vital signs and/or the confirming of damage to the endotracheal mucosa was insufficient in an experiment. In order to adhere to these requirements, we examined the endoscope system.

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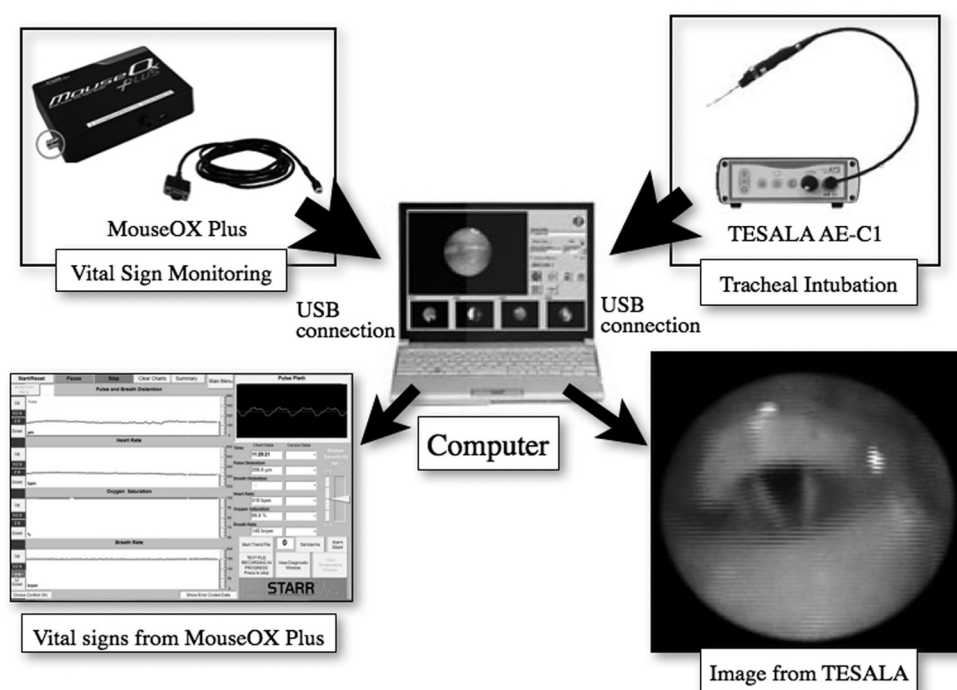


Fig. 1. Overview of mouse tracheal intubation system using endoscopic system, “TESALA AE-C1”. The data obtained from TESALA AE-C1 and MouseOx Plus are submitted into a computer and are displayed on the monitor both as the former endoscope image and as the latter graph of the status of the vital signs in real time.

## MATERIALS AND METHODS

**Animals and housing conditions:** Five males ( $29.3 \pm 2.80$  g body weight (b.w.)) (mean  $\pm$  SD) and 5 females ( $25.0 \pm 1.3$  g b.w.) of C57BL/6J mice were purchased from Charles River Laboratories Japan Inc., (Yokohama, Japan). Animal care and experimental procedures were approved by the Kyoto Sangyo University Committee for Animal Care and Welfare.

**Premedication:** In order to intubate an endotracheal tube, a mixture of three drugs described by Kawai *et al.* [13], which was composed of 0.3 mg/kg b.w. of medetomidine (Domitor®, Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan), 4.0 mg/kg b.w. of midazolam (Dormicum®, Astellas Pharma Inc., Tokyo, Japan) and 5.0 mg/kg b.w. of butorphanol (Vetorphale®, Meiji Seika Kaisha, Ltd., Tokyo, Japan), was used as premedication for immobilization, decrease in respiratory rate, muscle relaxation and sufficient analgesia of the mice. The name of it, “M/M/B: 0.3/4/5”, was used as a premedication for immobilization, decrease in ventilatory rate, muscle relaxation and sufficient analgesia of the mice. Atropine sulfate (ATROPINE SULFATE Injection 0.5 mg, Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan) diluted up to 50 times with sterilized distilled water, and it was also inoculated intraperitoneally to reduce the production of salivary and bronchial secretions and to reduce the risk of airway blockage.

**Endotracheal Intubation:** Endotracheal intubation was

performed using an endoscope technology from TESALA AE-C1 (AVS Co., Ltd., Tokyo, Japan. <http://www.avs.co.jp/animal/tesala/tesala3.html>) (Fig. 1). 1) Endoscope probe AE-F07070 (0.7 mm in outer diameter and 70 mm long) was used for capturing images and 20G endotracheal tube (48 mm long, KN-1008, Natsume Seisakusho, Co., Ltd., Tokyo, Japan) (Fig. 2). The endotracheal intubation procedure was accomplished by the following process: 1) After the injecting of M/M/B: 0.3/4/5 as the premedication and the diluted atropine sulfate, mice were placed on the intubation stand (KN-1014, Natsume Seisakusho, Co., Ltd.), and then, the laryngoscope (KN-1021, Natsume Seisakusho, Co., Ltd.) was inserted to guide the endotracheal tube insertion. 2) The endotracheal intubation was carried out while observing the images from the probe on the monitor to confirm both the position and the form of the larynx as well as the epiglottis. The probe covered with an endotracheal tube was inserted into the trachea through the larynx. 3) After the position at the tip of the tube was confirmed to be in the proper location, only the probe was removed and the tube was fixed to a position on the upper jaw using a 3–0 silk suture. 4) The intubated tubes were connected to the anesthetic circuit.

**Inhalation anesthesia of the mouse using a ventilator:** RC<sup>2</sup> Rodent Anesthesia System (VetEquip, Inc., Pleasanton, CA, U.S.A.) was used as a vaporizer, and as the anesthetic circuit, SLA Ventilator (Natsume Seisakusho, Co., Ltd.) was used as a ventilator for small animals. In addition, 100% O<sub>2</sub> gas was used as a carrier gas, and the ventilator was set as

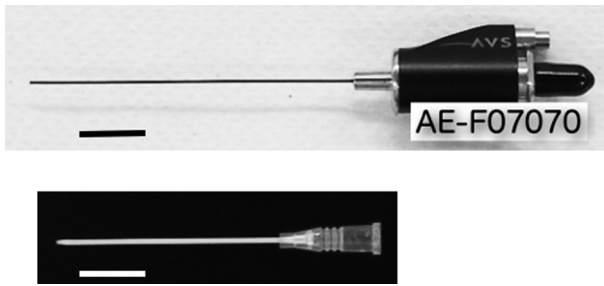


Fig. 2. Probe AE-F07070 and the tracheal tube for mouse tracheal intubation. The probe “AE-F07070 (0.7 mm in outer diameter and 70 mm long)” and 20G and 48 mm long tracheal tube were used for the mouse tracheal intubation. The probe was covered with the tube before the intubation and was then inserted. After clarifying the success of the tracheal intubation by the images on the PC monitor from the probe, only the probe was pulled out. The tube was fixed to the upper jaw using a 3–0 silk suture and then connected to the anesthetic circuit. Both bars=10 mm.

follows, 250 ml/ flow volume/min, 150 breaths/min (BPM), and 50% I / I+E (I: intake, E: exhaust). Airway pressure in the anesthetic circuit was also monitored continuously, and the alarm was set to sound when the pressure exceeded 1,500 pa [3, 8, 11, 14, 20, 22]. Finally, the inhalational anesthesia was carried out using isoflurane (Escain®, Mylan Seiyaku,

Osaka, Japan). In order to control spontaneous breathing of the mice, isoflurane was maintained at 5% concentration for 1 min after anesthetic circuit connection and then at 2% concentration for 14 min. Atipamezole (antisedan®, Nippon Zenyaku Kogyo Co., Ltd.), an antagonist to medetomidine, at a dose of 1.5 mg/kg b.w. (5 times of the given dose of medetomidine) was intraperitoneally inoculated to the mice injected with M/M/B: 0.3/4/5 immediately after the connection of the anesthetic circuit was made to eliminate the effect of M/M/B: 0.3/4/5. After finishing the isoflurane anesthesia, artificial respiration only by pure oxygen was continued. The mice were disconnected from the anesthetic circuit, once the reflection and spontaneous breathing had been stabilized, and their values of arterial oxygen saturation ( $SpO_2$ , %) displayed that of 90% or above. The endotracheal tube was removed when the reflection became stronger and spontaneous breathing was restored, and the animals were then returned to their cages. During the anesthesia, all mice were warmed on a hot plate while paying attention to the state of hypothermia of the mice until they were returned to their respective cages.

*Measurement and record of the mice's vital signs:* Vital signs of  $SpO_2$ , heart rate (HR, beat/min) and breath rate (BR, breath/min) were measured and recorded using MouseOx Plus® (STARR Life Sciences Corp., Pittsburgh, PA, U.S.A.) (Fig. 1). After shaving the murine hair of the neck using a hair clipper, the clip sensor was equipped onto the neck of

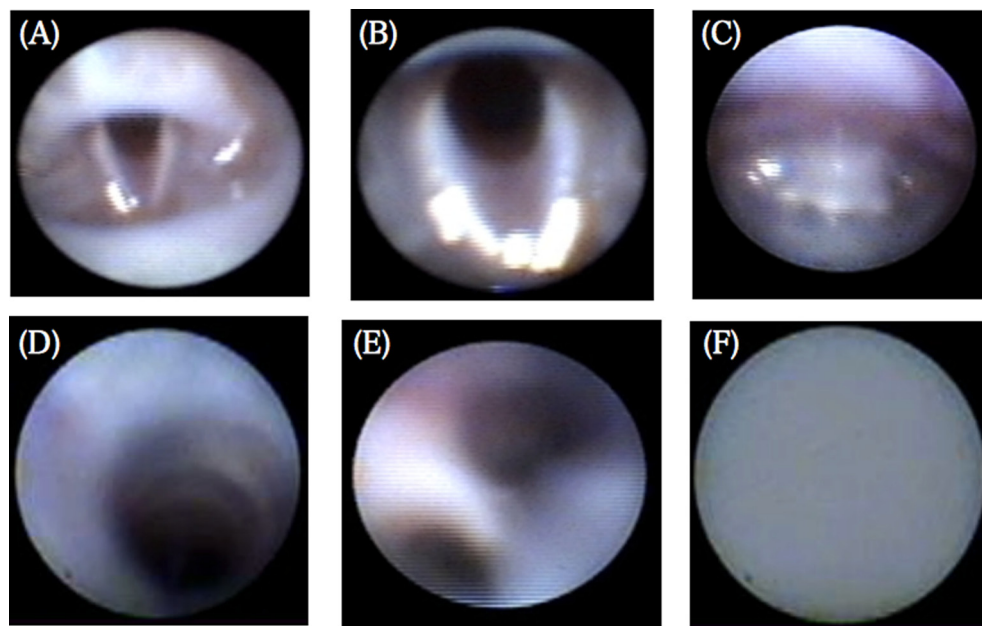


Fig. 3. Images in oral, trachea and esophagus from TESALA AE-C1. (A) Posterosuperior view of larynx with opened epiglottis<distal> (B) Posterosuperior view of larynx with opened epiglottis<proximal> (C) Posterosuperior view of larynx with closed epiglottis (D) Inside view of trachea (E) Inside view of bronchus from trachea (F) View of incorrect intubation into esophagus. (A) was common at the beginning of the tracheal intubation. (B) could be observed before the intubation to the trachea. (C) was occasionally seen though. (D) was confirmed when the intubation succeeded, and (E) was able to be also observed by advancing the probe. (F) was confirmed when the probe was mis-inserted into the esophagus.

Table 1. Result of the mouse tracheal intubation using TESALA

ID	Body Weight (g)	Sex	Kind of premedication	Time until success of intubation (sec)	Existence of troubles after intubation
3M-1	34.2	♂	M/M/B: 0.3/4/5	10	Success of the tracheal intubation without trouble
3M-2	28.1	♂	M/M/B: 0.3/4/5	10	Success of the tracheal intubation without trouble
3M-3	27.1	♂	M/M/B: 0.3/4/5	40	Success of the tracheal intubation without trouble
3M-4	28.8	♂	M/M/B: 0.3/4/5	30	Success of the tracheal intubation without trouble
3M-5	28.1	♂	M/M/B: 0.3/4/5	40	Success of the tracheal intubation without trouble
3M-6	24.3	♀	M/M/B: 0.3/4/5	30	Success of the tracheal intubation without trouble
3M-7	26.1	♀	M/M/B: 0.3/4/5	30	Success of the tracheal intubation without trouble
3M-8	24.1	♀	M/M/B: 0.3/4/5	50	Success of the tracheal intubation without trouble
3M-9	25.4	♀	M/M/B: 0.3/4/5	60	Success of the tracheal intubation without trouble
3M-10	22.7	♀	M/M/B: 0.3/4/5	50	Success of the tracheal intubation without trouble

M/M/B: 0.3/4/5 was composed of medetomidine (0.3 mg/kg) + midazolam (4.0 mg/kg) + butorphanol (5.0 mg/kg), respectively. M/M/B: 0.3/4/5 was intraperitoneally injected as a premedication.

the mouse to record SpO<sub>2</sub>, HR and BR.

**Statistical analysis:** Statistical analysis was conducted using JMP software (SAS Institute Inc., Cary, NC, U.S.A.) for analysis. Differences on SpO<sub>2</sub>, HR and BR between male and female were analyzed using the unpaired student's *t*.

## RESULTS

The endoscopic figures of the endotracheal intubation process from TESALA AC-1 were indicated in Fig. 3. When the probe-covered endotracheal tube was inserted into the larynx, the images of a closed or opened epiglottis were shown on the monitor of TESALAS AE-C1 system. The opened epiglottis could be confirmed by moving the larynx according to breath, and this made it easier to insert the endotracheal tube into the trachea (Fig. 3A and 3B). When the epiglottis was closed, it was more difficult to insert the tube, but it could be opened by lifting up the epiglottis gently using the tip of the probe, TESALAS AE-C1 system (Fig. 3C).

The endotracheal tube was intubated through the opened epiglottis, and the inside of the trachea can be then viewed as the shape of bellows (Fig. 3D). When the origin of the bronchus is confirmed by deeply inserting the probe, focus is needed to avoid any damage of the inside wall of the trachea or breaking of the bronchus. (Fig. 3E). Furthermore, the smooth and flat image indicated that the tube was mistakenly intubated into the esophagus because of the clinging of mucosa on the tip of the probe (Fig. 3F).

Results of the endotracheal intubation are shown in Table 1. All mice injected intraperitoneally with M/M/B: 0.3/4/5 were successfully treated with the endotracheal intubation without any ventilatory arrest or difficulty. Then, all mice were intubated and connected to the anesthetic circuit. The time of intubation was 10–60 sec (mean  $\pm$  SD: 35.0  $\pm$  16.5 sec) in the 10 mice injected with M/M/B: 0.3/4/5, and none suffered from dyspnea. There was no difference between the amount of time taken to complete the intubation process between male and female. The results of vital signs measured are shown in Fig. 4. In all mice, the values of SpO<sub>2</sub> reached up to 95% or more within 1 min after the anesthetic circuit connection was established and those values continued for

15 min. The heart rate (HR) and the breath rate (BR) were stable at 15 min after the connection of the circuit (489.4  $\pm$  219.4 beat/min and 185.1  $\pm$  101.2 breath/min). Although the BR was a little higher than the rate set by the ventilator at 150 times/min, they appeared to be relatively stable.

## DISCUSSION

The methods of anesthesia for mice are classified roughly into 2 types, injection anesthesia and inhalational anesthesia. The latter is also classified into 2 types, the mask anesthesia and the anesthesia, which uses the ventilator. The mask anesthesia can be operated without the requirement of an expensive and complicated device, but sometimes laboratory animals can die from breathing difficulties because the gas exchange depends on spontaneous breathing. On the other hand, though the anesthesia using the ventilator requires endotracheal intubation, the risk of breathing trouble is quite low because artificial breath is mechanically controlled by the ventilator. Hence, the anesthesia using the ventilator is useful not only in general anesthesia, like large laboratory animals, such as dogs, pigs and monkeys, but also in mice as well as general anesthesia for rodents. It can be used for time-consuming surgeries and open heart surgeries. The endotracheal intubation is also applicable to mice for obtaining imaging data, such as, an MRI or CT. In this experiment, we introduced a new, easy and safe intubation method by using an endoscope system for small rodents. M/M/B: 0.3/4/5 was used as a premedication for 5 males and 5 females. Results of the endotracheal intubation showed that the new visible method was quick and safe (Fig. 3 and Table 1). Moreover, they also suggested 4 advantages; 1) Since the success or failure of intubation can be determined visually, it can be performed successfully, preventing the incorrect placement of intubation. 2) The damaging of the mouse respiratory system during the tracheal intubation can be discovered easily, because of image from the endoscope. 3) As a result, prevention is beforehand possible in the difficulty during the inhalational anesthesia. And 4) Additionally, this brings the practice of the animal welfare. The purposes of premedication are to make the animals sedative, analgesic and

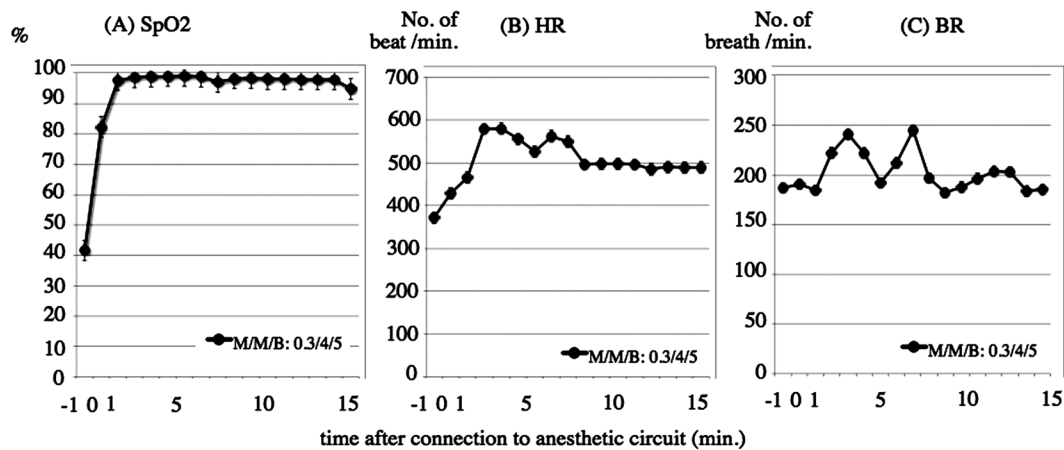


Fig. 4. Vital signs during inhalation anesthesia of tracheal-intubated mouse in groups both pentobarbital sodium injected and M/M/B: 0.3/4/5 injected mice. (A) SpO<sub>2</sub>; arterial oxygen saturation (%), (B) HR; heart rate (beat /min), (C) BR; breath rate (breath /min) (A) The values of SpO<sub>2</sub> reached to 95% or more from 1 min after the anesthetic circuit connection until 15 min, and no significant difference was observed in the values of SpO<sub>2</sub> between both groups ( $P>0.05$ ). (B) However, the HR values of M/M/B: 0.3/4/5 injected group were bigger than those of pentobarbital sodium injected group, and no significant difference was observed between the 2 groups. (C) The BR values of M/M/B: 0.3/4/5 injected group showed relatively stable except at 2 to 4 min and at 7 min, though they were a little bigger than that of the set by the ventilator (150 times/min). However, the low values of pentobarbital sodium injected group were shown to be significant from 8 min until the end of the anesthesia.

muscularly-relaxed for easy tracheal intubation before the inhalational anesthesia. [4, 6–8, 16, 22]. However, if muscle relaxation is too strong, sometimes the animals will die of dyspnea. Therefore, the choice of anesthetics, the dosage and the injection route of the premedication greatly influences the success or failure of the tracheal intubation method. According to the description for M/M/B: 0.3/4/5 by Kawai *et al.* [13], the time until immobilization was  $2.67 \pm 0.58$  min, and the time required by an anesthetic stage was  $5.67 \pm 2.31$  min. Since the mice became immobile about 3 min after the injection of M/M/B: 0.3/4/5 in this experiment as well as Kawai's report, the shave had begun at 3 min after the premedication and the tracheal intubation had begun at about 8 min. Moreover, all mice inoculated with M/M/B: 0.3/4/5 showed no breathing trouble during and/or after the tracheal intubation. In addition, vital signs in Fig. 4 showed that SpO<sub>2</sub>, HR and BR were comparatively stable from the beginning to the end [1, 5]. Thus, we think M/M/B: 0.3/4/5 is one of the most suitable premedication agents for murine tracheal intubation and following isoflurane anesthesia. Mice were intraperitoneally injected with atipamezole immediately after the connection to the circuit. This drug is an  $\alpha_2$ -antagonist and a specific reversal agent to medetomidine. We think that atipamezole suppressed the effect of the medetomidine in M/M/B: 0.3/4/5 and made the BR of mice injected with M/M/B: 0.3/4/5, stable and balanced during the isoflurane anesthesia. More studies are needed to outline the effects of atipamezole on premedication and inhalational anesthesia. In this experiment, we demonstrated a new, easy, quick and safer tracheal intubation method of inhalational anesthesia for mice using the exceptional endoscope system, TESALAS AE-C1 and premedication of M/M/B: 0.3/4/5.

With the widespread use of this method, it would benefit not only for the research data, but also for caring for the well-being of the animal, as well as the three "R"s principle for experimentation that is being performed on animals.

**ACKNOWLEDGMENTS.** This work was supported by a grant from "Kyoto Sangyo University Research Grants: No. C1102". I would like to thank Mr. Masayuki Hashiura, Hakubatec Lifescience Solutions Co., Ltd., Japan, for scientific and technical guidance and information.

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