**Journal of the American Association for Laboratory Animal Science**

Volume 58, Number 2, March 2019

**ORIGINAL RESEARCH**

***Husbandry***

**Peveler et al.** [**Effects of Various Commercially Available Enrichment Options on Handling and Chronic Stress Markers in Female ICR Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00001)**, pp. 119-125**

Domain 4:  Animal Care

Primary Species:  Mouse (*Mus musculus*)

SUMMARY: This study was done using female ICR mice to determine if singly-housed mice could benefit from 1 of 10 different type of environmental enrichment strategies, as compared to be housed as a pair or alone. They hypothesized that singly-housed, unenriched mice would demonstrate behavioral and physiologic evidence of being stressed, that those pair housed would be the least stressed, and that singly-housed, environmentally-enriched animals would fall somewhere in between. Enrichment strategies that were evaluated included those that would encourage natural behaviors including foraging, exercise, sheltering, and socialization. Treatment groups were observed for a period of three months and behavioral and physiologic parameters were evaluated using measurements of body weight, CBC, organ weights, and behavior was evaluated using scale criteria (0 – enrichment item shows no sign of being used, 1 – enrichment item shows sign of use, and 2 – enrichment item is currently being used). Results indicated that there was no significant difference between the singly-housed mice as compared to the pair-housed mice and those with no enrichment, in terms of physiologic parameters, except for the body weight (increased in pair-housed mice and mice provided cotton squares and lowest in mice that were assigned to running wheels).

QUESTIONS

1. Which of the following nesting material has been associated with eye lesions in nude mice?
   1. Enviro-Dri
   2. Enviro-Pak
   3. Nestlets
   4. Facial Tissue
2. True or False. In some strains of mice, cage dividers and shelters have induced overt aggression in groups of males, resulting social stress and injury.
3. True or False. In this paper, the running track was thought to decrease weight due to the stress induced by this novel item.

ANSWERS

1. c
2. True
3. False

**Lim et al. Retrospective Analysis of the Effects of Identification Procedures and Cage Changing by using Data from Automated, Continuous Monitoring, pp. 126-141**

Domain 4: Animal Care, Task #T1, Knowledge husbandry (housing)

Primary Species:  Mouse (*Mus musculus*)

SUMMARY: This was a retrospective analysis on the effects of 2 common identification procedures (i.e. ear tagging & tail tattoo without anesthesia) and the effects of cage changing evaluating motion and respiratory rate of mice since it is suggested a decrease in motion and increase in respiratory rate can indicate pain or discomfort. This paper also evaluated 3 common mouse strains (i.e. C57BL/6J, BALB/cJ, and C3H/J), sex and age of mice after cage change as well as timing of cage change (i.e. 6am-2pm early vs. 5pm late). The final analysis was evaluating motion and respiratory rate of B6 mice on cuprizone-diet to induce Multiple sclerosis with every other week cage changes during a 41 day period. Some mice were singly housed in Vium Digital Smart cages which continuously monitor motion or activity levels as well as respiratory rate. They found there was a modest elevation in breathing rate after tattooing compared to ear tagging which may suggest that tattooing is more invasive between the two procedures. Compared to animal identification procedures, cage changing lead to a 3-fold increase in daytime motion compared to baseline with mild increase in nighttime motion (1.5 fold). These changes lasted at least 2 days post-procedure.  BALB/cJ mice showed heightened responses compared to C57BL/6J & C3H/J strains and specifically BALB/cJ male mice. Age did not seem to affect the rodents’ response. They found that responses to cage changing were strain and sex dependent; however the time of day at which cage changing was performed affected the response. Cage changing conducted late during the day (at the beginning of the rodent’s light cycle at 1700) reduced daytime motion on the day of the procedure compared to cages changed earlier in the day (0600-1400); however the effects of late cage changing appeared to be prolonged in males resulting in higher daytime motion. Regardless of the time of the procedure, mice modify their behavior patterns in response to a cage change.

As expected, mice fed cuprizone showed decrease in spontaneous activity compared to mice fed control chow; however there were periods of apparent ‘recovery’ when disease animals displayed similar levels of activity compared with the controls. These periods coincided with increases in daytime motion after cage changing. These results reveal inadvertent effects of cage changes on collection and interpretation of study data.

Increases in motion and breathing rate after cage changing may be indicative of hyperactivity, increase energy expenditure or acute stress from exposure of novel environment and removal of prior olfactory cues. This study suggests that it takes approximately 2-4 days for mice behavior and physiology to return to levels before cage changing occurred.

QUESTIONS

1. Previous research supports that husbandry procedures are associated with a number of behavioral and physiological effects in rodents. Which of outcomes listed below, is not linked to husbandry effects, such as cage changing, in rodents?

a. Increases in heart rate and mean arterial pressure

b. Aggression among male cage mates

c.  Decrease in scent marking behavior

d.  Disruptions in sleep and circadian rhythms

e.  Elevations in stress responses

2. Olfactory cues are used by rodents to \_\_.

a.  Establish a bathroom area and zone of aeration

b. Establish territory and recognize cage mates

c.  Inhibit grooming behavior and play

d.  Inhibit vocalization and play behavior

3. True or False. Cage changing has no effect on behavior or physiology of rodents.

 ANSWERS

1. c – all other statements have been reported as an outcome associated with husbandry

2. b

3. False – cage changing does have an effect on behavior and physiology of rodents

**Roughan and Sevenoaks.** [**Welfare and Scientific Considerations of Tattooing and Ear Tagging for Mouse Identification**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00003)**, pp. 142-153**

Domain 2: Management of Pain and Distress; Task 2: Minimize or eliminate pain and/or distress

Primary Species:  Mouse (*Mus musculus*)

SUMMARY: When considering animal welfare and the scientific costs of identification methods for mice, a lack of consensus still remains. The goal of the present study was two-fold: to evaluate the effect of tunnel-handling as compared to tail-handling, and to assess the welfare costs of tattooing as compared to ear tagging. Tattooing caused greater agitation than restraint alone. Postprocedural activity was no different between the ear tagged and tattoo groups. Grooming was not more frequent between the groups; however time spent grooming was prolonged in the tattoo group. There was no overall difference in grimacing between the tattooed mice and those that were only restrained by the tattoo machine. Interestingly, mouse grimace scale (MGS) scores decreased following tattooing, however they increased following restraint. Ear tagged mice grimaced more than those that were tattooed, and appeared to have a more severe grimace compared to those that were restrained.  Overall, male mice grimaced more than females. MSG scores were higher in tail-handled mice as compared to tunnel-handled mice. Mice that had been tunnel-handled remained less fearful than those that were tail-handled, approaching and interacting with the assessor's hand more frequently.

The tails of tattooed and tunnel-handled mice were more inflamed than animals that were restrained or tail-handled, respectively. The tails of male mice were more inflamed than those of female mice. A regression analysis showed degree of agitation and abnormal grooming can be predictive of the level of inflammation after tattooing or restraint procedures. All tattoos were read correctly without being handled by the assessor. Ear tag numbers were misread 45% and 25% of the time by the experienced and novice groups respectively.

Results of this study suggest that tattooing was not more painful than ear tagging but was more stressful, however the anxiogenic effects of tattooing were longer-lasting than those of restraint or ear tagging. The long-term benefits of tattooing, reduced chances of misidentification and decreased handling, balances concerns regarding increased stress, anxiety, or pain associated with tattooing. Based on these findings, researchers concluded that there is little justification for choosing ear tagging over tattooing.

QUESTIONS

1. Approximately what percentage of time are ear tags misread by experienced users?
2. T/F: Tail tattooing has been proven to be more painful than ear tagging.
3. T/F: Tails of tunnel-handled mice were more inflammation than those that were tail-handled

ANSWERS

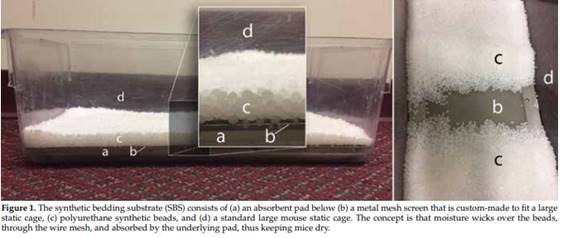
1. 45% of experienced users misread ear tag numbers
2. False - tattooing was proven to be more *stressful* but not more painful
3. True - however, the group suggests that tunnel-handled mice may have improved circulation as compared to tail-handled mice, thereby increasing luminol distribution through lack of handling.

**Bellin et al.** [**Evaluation of a Synthetic Bedding Substrate for Mice (*Mus musculus*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00004)**, pp. 154-159**

**Domain 4: A**nimal care.

Primary Species:  Mouse (*Mus musculus*)

**SUMMARY:** Bedding is one of the most important factors in a laboratory animal’s environment. Many of the common products today create a substantial amount of dust that can cause respiratory damage to the animals and technicians as well as contaminate the eyes of mice. Typically, 10% of mice received from vendors have some type of background of corneal injury that precludes use in ocular studies, with particular from bedding coming in contact with the eye to be the leading suspected cause. Synthetic bedding substrate (SBS) was developed to be particulate and contamination free and consists of an absorbent pad (cellulose-isopad) that is overlaid with fine stainless-steel mesh which is covered with irregularly shaped biocompatible polyethylene shot (beads; figure 1). This study compared living conditions between SBS (n=50) and standard woodchip bedding (n=50) in female 3-week-old BALB/cAnNCrl mice. Mice were weighed, assigned a health score (0 normal – 4 extremely abnormal) and underwent slit lamp examination at endpoint ~ 10 weeks.



**Results:** Mice on standard woodchip bedding had slight, but significantly higher weight gain on average than SBS group. Mice on standard bedding received a total score of 0, while mice on SBS had scores from 1-4 throughout study. Mice on SBS bedding were wet and unkempt so cage change frequency increased to every other day. By the end of the study, SBS mice were at a health score of 4 due to score of 1(unkempt), 1 (less mobile and no nesting), and 2 (moderate change in reaction to stimuli/were easily caught). In contrast, mice on normal bedding were well groomed and spent much of their time in or on the bedding. At completion of slit lamp, SBS mice were placed on standard bedding and began to elicit more normal behaviors. SBS did not control moisture well as large urine drops were observed on bedding. On slit-lamp examination, no significant differences in corneal damage were noted between the 2 bedding groups.

**QUESTIONS**

1.  What is one of the most important characteristics of rodent bedding?

a.   Texture

b.  Ability to provoke natural behavior

c.  Moisture absorbency

d.  Cost-effective

2.  Corneal opacities and anterior polar cataracts are a common developmental defect in inbred \_\_\_\_\_\_\_\_\_ mice.

a.   C57Bl

b.  BALB/c

c.    Swiss Webster

d.  129

e.   DBA

ANSWERS

* + 1. c
    2. a

**Bloomsmith et al.** [**Survey of Behavioral Indices of Welfare in Research Chimpanzees (Pan troglodytes) in the United States**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00005)**, pp. 160-177**

Domain 4 (Animal Care)

Tertiary Species: Other Nonhuman Primates

SUMMARY: Between 2015-2017, a written survey regarding housing and behavioral incidences of welfare was distributed to each chimpanzee research facility in the United States. The survey addressed an extensive range of topics including age, sex, rearing history, outdoor access, enclosure substrate, tool use, nesting, copulation, grooming behavior, abnormal behaviors and use of training (positive reinforcement training). Data were compiled on 701 chimpanzees and discussed in reference to recommendations made by the NIH Working Group on the Use of Chimpanzees in NIH-supported Research and previously published data from zoo-housed chimpanzees.

Broadly, the survey found that chimpanzees at U.S. research facilities are socially housed, have daily access to outdoor spaces and have been trained using positive reinforcement techniques to cooperate with husbandry and veterinary procedures. 45.4% of surveyed chimpanzees displayed all four key species-specific behavior (tool use, nest building, copulating, grooming initiation). 37.1% of surveyed chimpanzees displayed some form of abnormal behavior and many of these behaviors were influenced by early rearing history (early removal from mother, isolated rearing). Authors concluded that further in-depth comparisons of welfare-related information across zoos, sanctuaries, research facilities and privately owned facilities will continue to improve welfare of captive chimpanzees in the United States.

QUESTIONS

1. What are the 6 research facilities in the United States that house chimpanzees?

2. According to the NIH Working Group Report, “chimpanzees must have the opportunity to live in sufficiently large, multi-male, multi-female social groupings, ideally consisting of at least \_\_\_\_ individuals”.

3. Positive reinforcement training (PRT) is a well-established refinement in chimpanzee care. Which of the following has/have been documented as benefit(s) of using PRT in captive chimpanzees?

a. Improved the ease and efficiency of management

b. Reduced chimpanzee distress related to research and veterinary procedures

c. Decreased abnormal and stress-related behaviors

d. Reduced aggressive behaviors

4. T/F: The first year of life has profound and lasting influences on multiple behavioral measures of chimpanzee welfare.

ANSWERS

1. Yerkes National Primate Research Center, the Southwest National Primate Research Center, the Keeling Center, the New Iberia Research Center, the Alamogordo Primate Facility, and the Language Research Center at Georgia State University

2. 7 (According to current survey information, only 36.5% of chimpanzees lived in groups of 7 or more)

3. All are correct. The current survey reported that 72.2% of chimpanzees cooperate with receiving an injection on anesthetic and 69.5% present body parts for examination suggesting PRT is well-established across U.S. chimpanzee research facilities and effective on a widespread basis.

4. True

***Management***

**Zhang et al.** [**Influence of Rater Training on Inter- and Intrarater Reliability When Using the Rat Grimace Scale**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00006)**, pp. 178-183**

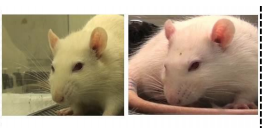
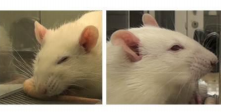
Domain2: Management of Pain and Distress

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY:This group aimed to examine what level of training is required to attain proficiency in using the Rat Grimace Scale (RGS), and they hypothesized that training would improve interrater reliability. Two unique sets of training images (42 and 150 images), prepared from acute pain models, were scored by two groups of participants using the RGS. The first group (n = 4), had no experience with the RGS, but were trained by an experienced rater and accompanying training manual. This group participated in group discussions with the experienced rater regarding their scoring decisions. Four years later, trainees in the first group re-scored the 150 images. A second group of raters (n = 8), received no training from the experienced rater, did not participate in group discussions, but did have access to the training manual.

The first (trained) group experienced an increase in interrater reliability between the 42 and 150 image sets, as well as improvement 4 years later. Orbital tightening was most consistently appropriately scored, and whisker position was least consistently appropriately scored. Interrater reliability in the no-training group did not improve from the 42 to 150 image sets. The results from this paper suggest that scoring reliability is minimal when raters review training manuals and score images without the opportunity for feedback/discussion. Improvement did occur when feedback and discussion with an experienced rater was included.

QUESTIONS

1. What are the scoring parameters when using the RGS?
2. Indicate the appropriate score for the following images using the RGS:
   1. 
   2. 
   3. 

ANSWERS

1. “All faces are coded for the presence/intensity of the specific facial Action Units (AU), relative to the status of the region of the baseline prototype” (nc3rs.org.uk)
   1. 0 = AU is not present
   2. 1 = AU moderately visible
   3. 2 = AU pronounced
2. Using the RGS:
   1. 1
   2. 2
   3. 0

**Morrow and Wiler.** [**Ammonia Measurement in the IVC Microenvironment**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00007)**, pp. 184-189**

Domain 4: Animal Care

Primary Species:  Mouse (*Mus musculus*)

**SUMMARY:** Review of 38 publications that compared the technology and methodology used to measure ammonia within the microenvironment of mouse cages.

Ammonia Sampling Technology: This technology is used to identify what gas is present, its concentration range, if the gas is dangerous to humans, and what PPE is required. There 4 different types - NOx gas analysis, Photoionization detection, colorimetric analysis, and electrochemical sensors (this one has not been used yet to detect ammonia in an IVC).

Colorimetric Analyzers: Portable, convenient, lower cost, and easy to use. Resolution of 1ppm and accuracy of 15-20%. Available as a manual pump or Drӓger measuring chip. No capability for real-time data logging and not designed to provide quantitative or highly accurate ammonia concentration readings. Do not require calibration.

NOx Analyzers: Designed to measure specific toxic gases according to specific fingerprint characteristics of target gases. Sensor range of 0-10,000ppm, resolution of 0.2ppm, less than 15sec response time, and limited cross sensitivity to other gases. Limited use due to capital cost and minimal portability.

Photoionization Detector PID: Portable and capable of measuring a wide concentration range 0-1000ppm. Resolution of 0.1ppm and response time of 3-5secs. Nonspecific to individual toxic gases and have negative interactions from cross-sensitivity with other gases.

Electrochemical Sensors: Designed to measure specific gases. Range of 0-100ppm, resolution of 1 ppm, 150 sec response time, and limited cross sensitivity to other gases. Fixed life expectancy of 1-3yrs and low maximal over-range rating (300ppm is the ammonia level considered to be dangerous to humans).

Combined Measurement Methods: PID and electrochemical sensors are often combined into a multi gas detector and provide a simultaneous measurement of the environment by 2 different sensor types. This is advantageous because it increases accuracy, sensitivity, and range. None of the 38 publications used this method.

Environmental Parameter and Sampling Methodology: Sampling methodology influences ammonia measurements and reliability of the sensor depends on ensuring the gas sensor is providing accurate readings. Sensors can become saturated and lose sensitivity over time. Calibration of sensors should be performed per manufacture guidelines and a daily bump tests should be performed to ensure that the sensor is within range. Ammonia absorbs into most surfaces and can cause positive and negative artifacts. Thus the air pump rate (300-1000ml/min) and reactivity to materials are important to know so that adequate air flow is reaching the sensor. Teflon PFA and stainless steel decrease the surface adhesion of ammonia and increases the accuracy of the sensor reading. Airflow dynamics and activity of the cage occupants affect the distribution of ammonia within IVC. Collection point of air sample, cage population, activity levels, temperature, and humidity are all variables that can affect an ammonia reading. Most of the publications used a port at the front of the cage or outside of the cage using a vacuum pump to obtain the air sample.

Conclusion: Currently no standardized methodology to assessing ammonia concentrations inside the cage. The review found marked variations in regard to technology used and sampling methods making it hard to reproduce these studies across institutions. Data from one sensor cannot be directly compared with data from another sensor due to differences in accuracy, sensitivity, and analytical capabilities. An exception to this would be the multi gas devices but those require specific training and calibration. Variability in studies is also an issue since ammonia is highly reactive and the amount generated depends on pH, temperature, and dissolved salts. The review found a need to develop more reproducible experimental methodologies for measuring ammonia levels within the IVC microenvironment if ammonia levels are going to be used to indicate the quality of the environment within the cage.

**QUESTIONS**

1. What are the 4 ways to test for ammonia?

2. What is a bump test and why is it important?

**ANSWERS**

1. NOx gas analysis, Photoionization detection, colorimetric analysis, and electrochemical sensors

2. A bump test is used to test a gas sensor via exposing the sensor to a known concentration of a target gas to see if it is reading appropriately. If the reading is off then you know the sensor needs to be recalibrated

**Gorence et al.** [**Chemical Contaminants from Plastics in the Animal Environment**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00008)**, pp. 190-196**

Domain 4: Animal Care

SUMMARY: Control animal variability was noted in a research program investigating endocrine-disrupting chemicals. Damaged polysulfone caging, known to leach bisphenol A and bisphenol S with age, was originally hypothesized to be the source of animal contamination. Investigation into the timing of chemical leaching identified that bisphenol S was present on the surface of new polysulfone cages after only two handwashing events following placement into facility circulation with no grossly visible damage. This suggested cross-contamination of the new cages by old cages and other plastics remaining in circulation during the sanitization and sterilization process. The facility then replaced all animal housing components at the facility and the investigative staff purchased fresh breeding stock from the vendor. Following this change, meiotic recombination in control animals returned to normal, historical levels. The authors beg attention to detail in animal care to provide a stable environment under which research can be conducted and encourage the view that all animal-contact materials may serve as a source of unwanted contaminants.

QUESTIONS

1.   Which alternative material could be utilized by this program to eliminate leaching of bisphenol A and bisphenol S?

a.   Polyphthalate carbonate

b.   Polyetherimide

c.  Polyethylene terephthalate

d.  Polysulfone

2.   Which common component of natural-ingredient rodent diets must be limited to avoid interference with the uterotrophic assay published by the Environmental Protection Agency?

a.  Alfalfa

b. Bone meal

c.  Casein

d. Corn gluten meal

e.  Wheat middlings

ANSWERS

1.  c

2.   a

***Animal Health Surveillance***

**Zaias et al. Seroconversion of 1-year-old Mice to Murine Norovirus, pp. 197-200**

Domain 4: Animal Care

Primary Species:  Mouse (*Mus musculus*)

SUMMARY:Historical practice is to use younger animals (4-10 wks) as live animal sentinels as the immunologic response decreases over time in mice and people. Depending on the size of the institution, this can result in high yearly usage of sentinel animals. The authors set out to evaluate whether older animals (40-48 wks) would seroconvert to murine norovirus as well as younger animals after direct (gavage) and indirect (dirty bedding) inoculation. This was evaluated in B6 and BALB/c mice. Young B6 mice directly inoculated by gavage were used to produce MNV-positive dirty bedding. Testing began 8 weeks post-exposure. At 8 weeks, younger mice had less seroconversion (2/8 B6, 6/8 BALB/c) than older mice (8/8 B6, 6/8 BALB/c) after indirect exposure. This difference was eliminated by 10 weeks post-exposure when all mice had seroconverted. All mice seroconverted after gavage by 8 weeks. The authors conclude that dirty bedding sentinel mice can therefore be used up to 1 year of age and be bled quarterly, at least for MNV monitoring.

QUESTION

1. What are the clinical signs of MNV in mice?

ANSWER

1. Primarily subclinical. Immunocompromised mice may show weight loss, hunched posture, and pathology in the GI, liver, and immune system.

**Dafni et al.** [**The Likelihood of Misidentifying Rodent Pasteurellaceae by Using Results from a Single PCR Assay**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00010)**, pp. 201-207**

Domain 1: Management of spontaneous and experimentally induced diseases and conditions

Primary Species:  Mouse (*Mus musculus*)

SUMMARY:The rodent Pasteurellaceae are considered among the most prevalent opportunistic bacterial pathogens isolated from experimental mice*.  Rodentibacter pneumotropicus* and *R. heylii* (formerly [Pasteurella] pneumotropica biotypes *Jawetz* and *Heyl*, respectively) are most often associated with rodent infections. Associated clinical symptoms, in general, are few. However, infections in immunocompetent mice can cause perturbations that have the potential to confound experiments and can cause a greater concern in immunocompromised mice. Due to unclear taxonomy, precise identification remains of rodent Pasteurellaceae is challenging.

Detection typically relies culture isolation of samples (usually from the nasopharynx) and subsequent biochemical characterization of suspected colonies. Commercially miniaturized biochemical test kits are available, but these kits are optimized for human samples and do not include all Pasteurellaceae species. Matrix-assisted laser desorption-ionization-of-flight mass spectrometry (MALDI-TOF MS) might achieve enough specificity of identification. However, this method currently lacks appropriate murine datasets. Institutional and commercial databases can develop their own databases. Due to limitations of other methods, PCR- based assays are considered to be the most reliable option available. Most PCRs assays target the 16s rRNA gene. Some detect all rodent Pasteurellaceae, whereas others can differentiate between species. Alternatives extending beyond the 16S rRNA gene include development of specific PCR assays based on the *gyrB*gene sequence and subsequent restriction fragment length polymorphism analysis and a multiplex PCR assay based on the 16S-23S rRNA internal transcribed spacer region. This study describes the struggles encountered in identifying a strain of bacteria that was new to the facility that was suspected to be *R. pneumotropicus* or *R. heylii.*It also describes our efforts to reconcile discrepancies between results from two different test. Initially, the biochemical test kit (API 20 NE, bioMerieux) and the first BLAST hit of the 16S rRNA gene sequencing (GenBank accession no. JQ346058.1) suggested that the bacteria is R. pneumotropicus or R. heylii. However, the biochemical system used was known to be unreliable for identification of Pasteurellace. A closer look at BLAST results showed that a second hit identified the bacteria as Pasteurellaceae. Isolates of the unknown Pasteurllaceae strain, together with four other isolate types were submitted to service laboratories. Microbiology performed by both Lab 1 and Lab 2, results of PCR analysis performed by Lab 2, and sequencing data formed at Lab 3 confirmed the presence of Pasteurellaceae. In contrast, PCR analysis performed by Lab 1 identified *R. heylii.*RT-PCR assay combined with HRM analysis of a variable region in the 16S rRNA gene sequence distinguished between isolates according to sequencing results. The one exception was that one of the M. muris strains showed the same HRM profile as Pasteurellaceae in the amplified region. A major limitation of HRM is that an unknown specimen not included in the reference library might have the same value as a nonassociated reference strain and allow for incorrect identification of species. The multiplex PCR assay targeting the 16S–23S rRNA internal transcribed spacer sequences provided additional evidence, supporting the identification of the unknown bacteria as Pasteurellaceae. In contrast to the HRM assay, the multiplex PCR assay correctly identified both M. muris strains in concordance with 16S rRNA gene sequencing results. Based on the results, the authors concluded that the new bacteria was Pasteurellaceae species and not *R. pneumotropicus* or *R. heylii*. The case also supports the advice to use a combination of several methods to achieve correct identification of Pasteurellaceae.

Although more time-consuming and expensive compared with a single PCR analysis, sequencing of the 16S rRNA gene is considered the most reliable method for identification and differentiation of Pasteurellaceae. However, some caution should be used, because there may be misidentified sequences, as the authors believe they experience with one of the samples. Finally, the authors recommend that Pasteurellaceae be added to health monitoring systems.

QUESTIONS

1. [Actinobacillus] muris was reclassified as\_\_\_\_\_\_\_\_\_.
   1. *Rodentibacter pneumotropicus*
   2. *Rodentibacter* heylii
   3. *Muribacter muris*
   4. *Pasteurella pneumotropica*
2. Detection of Pasteurellaceae usually relies on culture isolation of samples from:
   1. Rectum
   2. Pelt
   3. Nasopharynx
   4. Conjunctiva

ANSWERS

* + - 1. c
      2. c

**Ragland et al.** [**PCR Prevalence of Murine Opportunistic Microbes and their Mitigation by Using Vaporized Hydrogen Peroxide**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00011)**, pp. 208-215**

Domain 4: Animal Care

Primary Species:  Mouse (*Mus musculus*)

SUMMARY**:** Recent developments of immunodeficient mice namely: N1 (*Foxn1)*: athymic T-cell-deficient nude mice; C.B-17-Prkdcscid: T-and B-cell receptor rearrangements; NOD/LtSz-*scid*/IL2Rnull(NSG) : lack mature lymphocytes, NK cells, and IL2 receptor -chain; have allowed for more studies in human cancer treatment.

Because of the demand for more immunodeficient mice, Individually Ventilated Caging (IVC) has become commonplace and are very efficient at excluding pathogens and improving the microenvironments. Disease surveillance is an important component of maintaining healthy colonies and has become more sophisticated and in addition to sentinel programs where live mice are used to detect pathogens within a cage system, IVC exhausts are also now routinely tested as they are able to detect pathogens that are undetectable in sentinels.

It is no longer enough to exclude and monitor for known pathogenic invaders as the microbiota of mice become increasingly altered. Commensals or opportunistic organisms can become pathogenic when the normal microbiota is disrupted and form pathobionts creating dysbiosis thereby increasing morbidity and confounding study results.

Some examples of symbionts becoming pathogenic are:

* *Proteus mirabilis*+ *Klebsiella pneumoniae ­*= colitis in *Tbet-/-xRag2-/-*mice
* *Enterococcus*+ *Klebsiella oxytoca*= nephritis
* *Pasturella pneuotropica*= pneumonia in aged NSG (the most immunodeficient) mice
* *Staphylococcus xylosis* = dermatitis, abscess, cystitis
* *Corynebacterium bovis*= hyperkeratotic acanthotic dermatitis in nude mice
* *Pseudomonas aeruginosa,*β-hemolytic *Strep, Staph aureus, Pneumocystic carinii*normally considered commensals on humans or in the environment can become pathogenic in immunodeficient mice.

Problems: Current SOPs for handling, cleaning, and maintaining immunodeficient mice cannot prevent movement of potential pathogens when mice are moved around a facility for procedures; accumulation of opportunistic microbes in IVC exhaust plenums may be an additional source but has not been thoroughly investigated; some commensals that may become pathogenic are not currently monitored by commercial vendors.

This paper describes the testing of IVC exhaust to report the microbes detected in a SPF, viral antibody-free murine research facility to help facilitate appropriate health surveillance monitoring. They also describe an effective method for sterilizing IVC that eliminates opportunistic microbes in the exhaust plenums.

The three most commonly PCR isolated organisms were *Staphylococcus xylosus, Proteus mirabilis*and *Pasturella pneumotropica*biotype Heyl. The other five were *Klebsiella oxytoca, K. pneumoniae, Pseudomonas aeruginosa, Staph aureus, Pasturella pneumotropica*biotype Jawetz. The eight most commonly isolated organisms were all more readily detectable by PCR from exhaust samples. In fact, only 3 of the 15 microbes tested were detectable at all in murine specimens confirming the utility of IVC exhaust testing.

The best method for disinfecting the IVC was to connect the assembled IVC to the Air Handling Unit (AHU) and set on active-closed to draw Vaporized Hydrogen Peroxide (VHP) through the IVC manifolds and plenums. The authors suggest that this method of sterilization will significantly decrease the accumulation of opportunistic microbes protecting the quality of research generated from immunodeficient mice. In addition, semiannual sterilization will help prevent biofilms from forming. Routine testing for *Staph xylosus*will validate the sterilization efficacy since this is the most prevalent opportunistic microbe.

QUESTIONS

1. What bacterial organism is responsible for opportunistic outbreaks in immunodeficient mice colonies and causes hyperkeratotic dermatitis potentially invalidating studies using NOD and NSG mice?
2. What is the method by which VHP works and what is the concentration range necessary?
3. All of the following have been previously identified as prevalent opportunistic microbes EXCEPT?
   1. *Helicobacter spp.*
   2. *Corynebacterium bovis*
   3. *Proteus mirabilis*
   4. *Pasturella pneumotropica*
   5. All of the above

ANSWERS

1. *Corynebacterium bovis*
2. Converts 35% H2O2liquid to gas via flash vaporization which is then dispersed in a dry air stream onto all exposed surfaces. It is broadly antimicrobial and sporicidal at 150-400 ppm.
3. e

***Anesthesia***

**Bradley et al.** [**Intramuscular Administration of Alfaxalone Alone and in Combination for Sedation and Anesthesia of Rabbits (Oryctolagus cuniculus)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00012)**, pp. 216-222**

Domain 2: Management of Pain and Distress

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: The study compared the sedative, anesthetic, and cardiovascular effects of intramuscular alfaxalone alone (6 mg/kg), and with butorphanol (0.3 mg/kg); midazolam (1 mg/kg), dexmedetomidine (0.2 mg/kg); and both butorphanol and dexmedetomidine (0.3 mg/kg and 0.2 mg/kg respectively) in female New Zealand White Rabbits. In addition, a follow up study was conducted using dexmedetomidine (0.2 mg/kg) intramuscularly and comparing sedative effects to data derived in the main study. All medications were administered in the caudal epaxial muscles. Following injection respiratory rate, heart rate, SpO2, rectal temperature, response to toe and ear pinch, and times to loss and return of righting reflex were recorded.

It was demonstrated that alfaxalone in combination with other sedatives provided a prolonged and reliable sedative effect, which in some combinations provided anesthesia. Administration of alfaxalone alone provided sedation for 40 minutes, while the addition of midazolam increased the duration of sedation by 25 minutes, the addition of dexmedetomidine increased sedation by 117 min, and the addition of butorphanol increased sedation by 8 minutes. The combination of alfaxalone-butorphanol-dexmedetomidine was the only combination to consistently produce a lack of toe and ear-pinch response, which lasted up to 1 hour in some animals and demonstrated the potential use for short, noninvasive surgical procedures. Alfaxalone provided smooth induction and recovery from sedation in each protocol, which was contrasted with the dexmedetomidine treated group which had several animals with strong startle responses, paddling, and multiple attempts to right themselves during recovery. Cardiovascular effects produced by the drug combinations were considered within acceptable limits for anesthetized rabbits, with the combinations using dexmedetomidine producing a greater decrease in heart rate.

QUESTIONS

1. What is the primary mechanism of action of alfaxalone and through which receptor does it produce its anesthetic/sedative effects?
2. What is the difference between additive drug effects and synergistic drug effects?

ANSWERS

1. Alfaxalone is a neuroactive steroid that is a positive allosteric modulator of GABA receptors, causing a hyperpolarization of the neuron and a strong anesthetic effect
2. An additive effect occurs when 2 drugs act on the same receptor site to produce a greater effect than the sum of their individual effects. Synergy occurs when 2 drugs act at different sites.

**Engel et al.** [**Regional Anesthesia for Dentistry and Orofacial Surgery in Rhesus Macaques (*Macaca mulatta*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00013)**, pp. 223-230**

Domain 2: Management of Pain and Distress

Primary Species: Macaques (*Macaca spp.*)

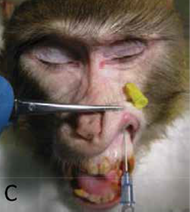
**SUMMARY:** Although regional anesthesia is commonly used for orofacial dental and surgical procedures in humans and companion animals there is a paucity of information describing techniques for performing regional blocks of the mandibular and maxillary nerves in rhesus macaques.   The authors of this manuscript describe anatomic landmarks for the maxillary and mandibular nerves and relevant branches; verify appropriate angles and depths for accessing the nerves; and determine appropriate injection volumes for saturation of specific areas of interest.  The authors conclude that regional anesthesia of the mandibular and maxillary nerves can be accomplished in rhesus macaques and applications include analgesia for dental procedures; treatment for trauma related injuries; and pain control during study related procedures.

**QUESTIONS**

1. Which of the following IS a branch of the maxillary nerve?
2. Greater palatine nerve
3. Nasopalatine nerve
4. Infraorbital nerve
5. All of the above
6. Which of the following IS a branch of the mandibular nerve?
7. Inferior alveolar nerve
8. Mental nerve
9. Lingual nerve
10. Long buccal nerve
11. Infraorbital nerve
12. All of the above
13. All but e
14. Which of the following rare, but serious complications have been reported in people who have had superior alveolar and inferior alveolar regional blocks?
15. Facial nerve palsy
16. Auditory and visual system defects
17. Abducens nerve palsy
18. All of the above
19. None of the above
20. What is structure is depicted by the arrow in the image below?



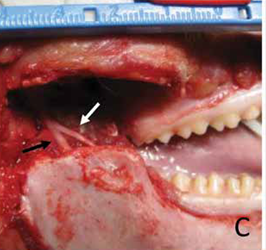
1. Pterygomaxillary fissure
2. Greater palatine foramen
3. Incisive foramen
4. Mental foramen
5. What technique is being depicted in the image below?



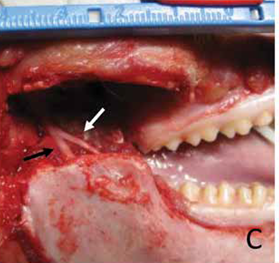
1. Maxillary nerve block
2. Greater palatine nerve block
3. Infraorbital nerve block
4. Inferior alveolar nerve block
5. What structure is depicted in the image below?



1. Infraorbital foramen
2. Mandibular foramen
3. Greater palatine foramen
4. Mental foramen
5. What nerve is depicted by the white arrow in the image below?



1. Lingual nerve
2. Inferior alveolar nerve
3. Greater palatine nerve
4. Nasopalatine nerve
5. What nerve is depicted by the black arrow in the image below?



1. Lingual nerve
2. Inferior alveolar nerve
3. Greater palatine nerve
4. Nasopalatine nerve

**ANSWERS**

1. d

2. g

3. d

4. a

5. c

6. d

7. a

8. b

***Experimental Use***

**Mason et al. Enrofloxacin Pharmacokinetics and Sampling Techniques in California Sea Hares (*Aplysia californica*), pp. 231-234**

Domain 4: Animal Care

Tertiary Species: Invertebrates

SUMMARY: Sea hares are commonly used for neurobiology (memory and learning) studies, can be susceptible to infection by the bacterium *Vibrio*spp. (common aquatic infection, can be zoonotic). In humans, enrofloxacin is used for treatment. The group evaluated administration of 5-6 mg/kg enrofloxacin being injected into hemolymph of 6 sea hares (with 2 additional controls). At various time points (up to 72 hrs.) post-injection, the group obtained small amounts of hemolymph using a 20 g needle. The group analyzed the samples for levels of enrofloxacin and ciprofloxacin (a common metabolite of enrofloxacin in some species) across those time points. Peak achieved concentrations were 3 ug/mL. For *Vibrio* spp., MIC(50) is variable, but in other species when a ratio of antibiotic concentration; MIC is performed, values range from a ratio 8 to 10 or more at minimum to be considered therapeutic. In this study, those ratios ranged from 8.6 to 119.8 in the individuals sampled, indicating levels of probable therapeutic success. Elimination half-life times were similar to those of other invertebrate species at an average of 20.3 hrs. Note that, according to other studies in invertebrates, concentrations achieved, elimination half-life, etc. may vary based on water temperature; these animals were maintained at 14-18 degrees C. Overall this paper indicates that enrofloxacin can be delivered to sea hares in concentrations that can be effective in other species at treating infections caused by *Vibrio*spp.

QUESTIONS

1.  Which of the following is the scientific name of the California Sea Hare, commonly used in neurobiological studies?

a.  *Caenorhabditis elegans*

b.   *Limulus Polyphemus*

c.   *Aplysia californica*

d.   *Doryteuthis pealeii*

e.   *Patiria pectinifera*

2.   In some species of mammals, birds, fish and reptiles, which of the following is a common metabolite of enrofloxacin that can be measured?

a.   Ofloxacin

b.  Ciprofloxacin

c.   Gentamicin

d.  Cyproheptadine

e.  Clindamycin

 3.  According to *The Guide for the Care and Use of Laboratory Animals*, the stocking density of an aquatic invertebrate species should not exceed the following:

a.  300 g/L of water

b.   600 g/L of water

c.  1 kg/5 L of water

d.   There are no specific recommendations

ANSWERS

1. c

2. b

3. d

**Emmer et al.** [**Evaluation of the Sterility of Press'n Seal Cling Film for Use in Rodent Surgery**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00015)**, pp. 235-239**

Domain 3: Research; K14. Aseptic requirements for performing surgery

SUMMARY: Press’n Seal is a commercially available domestic “cling film” product. Many institutions use it as an inexpensive alternative to surgical drape material, as Press‘n Seal is widely available, inexpensive, transparent, aids in maintaining body temperature and is self-adhesive. There is anecdotal evidence that some institutions have performed in-house microbial assessment. However, no investigations have been published in peer-reviewed journals re the sterility of the product, and the manufacturer does not guarantee sterility.

This study used ATP swabs and replicate organism detection and counting (RODAC) plates to estimate the bacterial load of Press’n Seal. ATP swabs were used to measure bioluminescence from cellular material present on the sampled surfaces. This assay detects any ATP present – whether in dead or live cells.

10 boxes of commercial Press’n Seal, from different manufacturing batches were used for testing. The material was stored to mimic the conditions of use – boxes were opened then left ajar on a shelf or countertop within an animal facility. Small sections of Press’n Seal were sampled from at different points on the roll.

The RODAC plates were applied 5 times to the section of roll under investigation and then cultured, checking for growth at 24, 48 and 72 hours. These were compared to control sampling from traditional drapes. Each roll was tested immediately after opening, on day 14 and day 28 after opening.

RODAC Analysis

All traditional drapes had no bacterial growth after 72 hours incubation. Some of the Press’n Seal samples did show bacterial growth.

* Day 0: 3/10
* Day 14: 0/20
* Day 28: 1/10

However, the distribution of colony forming units, and the type of organism identified on day 28 suggested investigator contamination. Therefore, the authors suggested that the decrease in positive results suggested the start of the roll (used on day 0) may have higher levels of contamination than further into the roll (used on subsequent dates)

ATP Analysis

Results showed higher levels of bioluminescence on the Press’n Seal compared to a traditional drape or a negative control, which increased over the 28-day period.

* Traditional drapes: 1.6+/- 0.51 RLU (reactive Light Units)
* Press’n Seal day 0: 2.3 +/- 0.91 RLU
* Press’n Seal day 14: 3.5 +/- 0.91 RLU
* Press’n Seal day 28: 6.4 +/ 0.54 RLU

However, as RODAC testing was performed before ATP swabbing, this may have generated false positive results, as swabbing a sterile RODAC plate generated bioluminescence as well.

The authors concluded that Press’n Seal is an acceptable alternative to traditional drapes for rodent surgery, although discarding the first 25cm of roll was a suggested precaution to avoid the potential contamination at the start of the roll

QUESTIONS (True or False)

1. The ATP Bioluminescence Assay measures the biological activity of cells present on a sample
2. Press’n Seal has been shown to be equally sterile to traditional drapes
3. The first portion of a roll of Press’n Seal should be discarded as it may have a higher level of bacterial contamination

ANSWERS

1. False
2. False
3. True

**Lee et al.** [**Capnography-guided Endotracheal Intubation as an Alternative to Existing Intubation Methods in Rabbits**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00016)**, pp. 240-245**

Domain 3

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: Various CNS depressants, including injectable anesthetics, inhalant anesthetics, neuroleptics, and narcotic analgesics can be administered alone or in combination to induce or maintain general anesthesia. However, inhalant anesthetics have gained popularity for extended procedures in rabbits. During inhalation anesthesia, placing an endotracheal tube provides several useful safety features over using a face mask. However, several distinctive anatomic and physiologic features in rabbits can make intubation a challenging and arduous task.

Capnography-guided intubation in rabbits has been reported as an alternative to existing intubation methods. It is a noninvasive monitoring technique using an infrared absorption measurement system to record change in partial pressure of CO2 within the breathing gases. It is useful in determining the correct placement of an endotracheal tube by detecting CO2 in exhaled gases. Because the presence of CO2 is a simple criterion for confirming successful endotracheal intubation, capnography was shown to be an effective tool for accomplishing endotracheal intubation in rabbits.

Despite its effectiveness, capnography-guided endotracheal intubation in rabbits was not widely adopted within clinical or laboratory veterinary medicine because it is not routinely available in most laboratory and clinical settings. In contrast, many veterinarians and veterinary practices now routinely use capnography to monitor their patients. This is due because equipment prices have fallen and technologic advances have allowed it to be incorporated into multiparameter monitors.

Capnography is divided into 2 main types: side stream and mainstream. The authors hypothesized that mainstream capnography would be advantageous over side stream capnography in reducing intubation times in rabbits. Moreover, they investigate the utility of capnography-guided intubation in rabbits as compared with conventional technique of laryngoscopy-guided endotracheal intubation in rabbits.

The investigation demonstrated that mainstream capnography was useful,  with significantly faster airway establishment than side stream intubation or laryngoscopy.

Mainstream capnography allows the intubator to position the endotracheal tube in response to real-time capnographic changes, thus allowing quick and accurate adjustment in endotracheal placement and advancement.

As an observation during preliminary trials, the authors explain that uncuffed endotracheal tube was associated with increased risk of failure during intubation than using a cuffed one.

QUESTIONS

1. Which one is false regarding the advantages of using endotracheal tube versus using a face mask in inhalatory anesthesia?

a. More efficient ventilatory support

b. Easier control of anesthetic depth

c. Improved scavenging of waster gases

d. Restricted oral space for dental work

e. Accurate capnographic monitoring

2. Which one of the following anatomical and physiological features of rabbits is false regarding the difficult task of endotracheal intubation?

a. Small oropharyngeal cavity

b. Narrow laryngeal glottis

c. Short incisors

d. Thick fleshy tongue

e. Epiglottis located dorsally over the soft palate

f. Frequent laryngospasm

3. Which methods have been previously evaluated to facilitate endotracheal intubation?

4. List at least 3 parameters used to assess the anesthetic depth for intubation.

5. Why mainstream capnography is better than side stream?

ANSWERS

1. d is false. Restricted oral space is a limitation of the use of mask.

2. c is false. The incisors are long

3. Endoscopy-guided intubation, videoendoscopy-guided intubation, blind intubation, retrograde intubation, direct laryngoscopy, capnography-guided intubation, nasotracheal intubation, and miniaturized light stylet-guided intubation.

4. Relaxed jaw tone, absence of righting reflex, and lack of voluntary movement in response to interdigital web pinching.

5. Mainstream capnography has the ability to sample, analyze, and display CO2 concentrations in real time.

**CASE REPORT**

**Collymore et al. Head Tilt in Immunodeficient Mice Due to Contamination of Drinking Water by *Burkholderia gladioli*, pp. 246-250**

Domain 1: Management of spontaneous and experimentally induced diseases and conditions

Primary Species:Mouse (*Mus musculus*)

SUMMARY: 37 clinical cases over 10 weeks in immunodeficient strains of mice (NSG, SCID Beige and Il2rg/Rag2-/-) housed in three different rooms presented with neurological signs (head tilt, circling, spinning when lifted by the tail and rolling), weight loss, hunched posture and lethargy. All clinically affected animals were euthanized and necropsy performed.

Purulent material was noted within the ear canal and liver in affected cases. 5 of 9 lesions cultured identified *Burkholderia gladioli.* Histological examination of lesions identified microabcesses with the presence of neutrophils and macrophages within the liver, spleen and kidney. *Burkholderia spp.* and *Ralstonia spp.* were cultured from within multiple parts of the automated watering system supplying the animal rooms.

Trimethoprim-sulphonamide was administered to a group of SCID beige mice housed with affected animals based on results of culture and sensitivity of those euthanized. This, however, did not appear to improve the prognosis for these animals and their clinical condition deteriorated to rolling and circling. Changes were made to the water quality assurance program including disinfection of the entire water distribution system with peracetic acid and hydrogen peroxide, autoclaving of whole racks, increased flushing regime of automated watering systems and the provision of autoclaved water in autoclaved bottles and sipper tubes for immunosuppressed animals. Following the implementation of all the changes to water quality assurance no further cases were identified.

QUESTIONS

1. Which of the following are methods to reduce microbiological load in drinking water supplies?

a. Reverse-osmosis

b. UV sterilization

c. Acidification

d. Chlorination

e. All of the above

2. *Burkholderia spp* are typically found living where?

ANSWERS

1. e

2. Water and soil

[**Association of Primate Veterinarians Guidelines for Jacket Use in Nonhuman Primates**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00018)**, pp. 252-253**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions  
SUMMARY

Purpose:These guidelines provide basic information for researchers, animal caregivers, veterinarians, and IACUCs to consider when assessing jacket use in NHPs. Association of Primate Veterinarians (APV) recognizes that jackets may be used to facilitate aspects of biomedical research projects. Jacket use should be justified and users should be adequately trained and familiar with potential complications.

Background:Jacketing NHPs can be stressful, so habituation is beneficial prior to study initiation. Jackets offer advantages in projects with animals instrumented with chronic, indwelling catheters for of blood samples collection or constant infusion of test articles. Other uses include protection of implants, fentanyl patches, surgical incisions, and traumatic wounds.

Guidelines

Protocol Review:IACUC should examine proposals to ensure jacket use has been described and justified and that suitable alternatives are not feasible. Each proposal should include a plan to habituate the animal and ensure the jacket’s integrity and cleanliness throughout the study (e.g. periodic changes, repairs as needed), and a description of parameters and schedule for monitoring the comfort and health of the animals. IACUC should determine if the jacket will interfere with social housing or implementation of the institutional environmental enrichment program.

Fitting the Jacket: Manufacturer may be consulted, but sizes are roughly based on species, body weight, and length. The fit is best customized with plastic zip ties, additional padding, and other adjustments. Jackets may produce discomfort, distress, or pain when not fitted properly. It should fit snugly and comfortably around the neck, thorax, abdomen, and armholes, and not restrict the animal’s normal movements or respiratory effort, or cause chafing, which may lead to skin lesions. Stretchable spandex or cotton undershirts may be used to reduce skin chafing from the jacket. Pockets and inserts may be added to customize jackets.

Jacket Habituation: Many NHPs habituate to a jacket within a few days. Signs of maladaptation include destruction of the jacket, poor appetite, depressed or aggressive attitude, decreased activity, self-injury, and stereotypic behavior. Previous studies have shown that fecal corticosterone levels return to baseline by day 3 after jacketing in rhesus macaques, but a long habituation period may be needed for long-term studies or for jackets with additional components (e.g. tether, swivel, or recording device). Species differences: baboons most tolerant, most macaques readily habituate, and African green monkeys often quickly destroy jackets. Sedatives, tranquilizers, or anxiolytics may be considered. Animals that fail to habituate should not be placed on a study that requires jacket use.

Monitoring Jacket Use: Examine jacketed animal regularly (at least weekly) to ensure proper fit, cleanliness, and health. Sedation may be necessary to allow a thorough exam and to make adjustments. An ill-fitting jacket can cause skin erosions. Minor lesions can be managed by readjusting the fit and providing padding. The most common locations for abrasions are underarm and shoulder areas, but all areas in contact should be examined. Severe wounds may require jacket removal or frequent sedations for wound cleaning and bandage changes. Jackets should be changed at least once monthly or when damaged or soiled. Jackets should be disinfected and laundered after each use.

Considerations for Long-Term Use of Jackets: Long-term use is often required for NHPs instrumented with externalized implants, such as an indwelling catheter protected by a tether.

Jacket Use for Socially Housed NHPs:Jacketed NHPs do not need to be routinely exempted from social housing. IACUCs should encourage use of new technologies to allow social housing of jacketed animals (e.g. telemetry signaling in group housed animals). Social housing of jacketed or instrumented NHPs must take into consideration exteriorized components that can be manipulated by a social partner.

Endpoints**:** Experimental endpoints should be clearly defined and include jacket use duration. Any maladaptation must prompt veterinary investigation, treatment, and further habituation or removal from the study.

Record Keeping: The clinical veterinarian should examine animals and review records regularly to ensure the health of each animal is monitored according to parameters outlined in the protocol or SOPs.

QUESTIONS

1. What does APV stand for?
2. What are examples of potential NHP jacket use in research?

ANSWERS

1.  Association of Primate Veterinarians

2.  Jackets offer advantages in projects in which animals have been instrumented with chronic, indwelling catheters for collection of blood samples or constant infusion of test articles. Other uses include protection of implants, fentanyl patches, surgical incisions, and traumatic wounds.

[**Association of Primate Veterinarians Food Restriction Guidelines for Nonhuman Primates in Biomedical Research**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000002/art00019)**, pp. 255-258**

Domain 3: Research; Primary species: nonhuman primates

SUMMARY: This paper was developed by the Associated of Primate Veterinarians to provide information on how to manage food restriction protocols used in NHP research.

Food Restriction As A Motivator:Limited access to food and creating a necessity to work for food is a common concept used with NHPs. The USDA and The Guide recommend that NHPs are provided an opportunity to work for their food through foraging and/or enrichment devices (puzzle feeders). Long term calorie restriction can significantly extend life span. When food restriction is for experimental purposes and not for the well-being of the animal, this must be approved by the IACUC. Researchers should first consider alternatives such as using a highly preferred food as positive reinforcement instead of restriction to reach the desired goal. If food restriction is necessary and justified, the least restrictive diet that will achieve the objections should be used.

Animal Selection:Animals should receive a full physical exam by a veterinarian prior to use in a food restriction protocol. Food restriction may be contraindicated in certain health conditions (i.e. diabetes, pregnancy, etc.). Not all animals will perform will with food restriction as a behavioral motivator. The tasks shouldn’t be too complex for the age or ability of the animal. An individual’s optimum food ration for growth, development and weight maintenance must be assessed prior to initiating food restriction. Food restriction involves quantifying the specific amount of food items an individual animal receives but this does not necessarily preclude the animal from social housing or interactions. Modular caging can be used to separate animals during feeding but allow for socialization the rest of the time.

Protocol Review And Justification:The use of food restriction can be a potentially highly stressful procedure.

Appropriate questions to consider during protocol review:

* Is food restriction essential for the research and scientifically justified?
* What is the schedule for food restriction and access?
* Will there be periods when the animal will have ad libitum access to food?
* How is restriction being accomplished?
* Has this been considered and justified?
* Does the investigative team have adequate previous experience with training monkeys on the task proposed?
* What are the endpoints for removal from testing and from the study?
* How will the animals be monitored?

Considerations When Initiating Food Restriction Protocols:Animals should be allowed free access to water. Animals must be acclimated to their new housing prior to beginning a food restriction protocol. Vitamins and supplements may be beneficial in maintaining body condition. The proportion of positive reinforcers which contribute to full ration shall be determined. Preferred reinforcers should be determined for each animal to decrease the level of food restriction necessary. Food restriction should be introduced gradually over several days to weeks. Problems may arise when abrupt changes to the diet are made.

Ongoing Monitoring During Food Restriction Studies:On a daily basis the following shall be monitored: the amount of food provided and how much is eaten at each meal, the amount of food reinforcements offered and consumed, and the behavior of the animal. On a weekly basis the following shall be monitored: Animals body weight. On a monthly basis the following shall be monitored: BCS. On a Semi-annually or annual basis the following shall be monitored: Serum chemistry and CBC.

Humane Endpoints:An animal shall be removed from a food restriction study if it has lost more than 15-20% of its optimal body weight, has an unsatisfactory BCS (<2.5/5), shows significant abnormal behaviors that have not improved with intervention, or has abnormal lab values.

QUESTIONS

1. True or False. Positive reinforcement with highly preferred foods shall always be considered prior to considering food restriction during a study.

2.   What is one thing that should be monitored on a weekly basis when an NHP is on a food restriction study?

3.   True or False. Food restriction should be introduced quickly to increase the motivation factor associated with this.

ANSWERS

1. True

2.   Body weight

3.   False