Journal of the American Association for Laboratory Animal Science
Copyright 2023
by the American Association for Laboratory Animal Science

Vol 62, No 3
May 2023
Pages 205–211

Assessing Reuse of Hypodermic Needles in Mice by means of Digital Imaging, Photomicrography, Bacterial Culture, Analysis of Nest Building, and Animal Vocalization

Terese E Bennett,[1,*] Jason Rizzo,[2] Sharon Yang,[3] and Edward Rosfjord[3]

Hypodermic needles are sometimes reused in animal research settings to preserve the viability of and to conserve limited quantities of injected material. However, the reuse of needles is strongly discouraged in human medicine to prevent injuries and the spread of infectious disease. No official guidelines prohibit needle reuse in veterinary medicine, although the practice may be discouraged. We hypothesized that reused needles would be significantly more blunt than unused needles and that reuse for additional injections would cause more animal stress. To test these ideas, we evaluated mice that were injected subcutaneously in the flank or mammary fat pad to generate cell line xenograft and mouse allograft models. Needles were reused up to 20 times, based on an IACUC-approved protocol. A subset of reused needles was digitally imaged to determine needle dullness based on the area of deformation from the secondary bevel angle; this parameter was not different between new needles and needles that had been reused 20 times. In addition, the number of times a needle was reused was not significantly related to audible mouse vocalization during injection. Finally, nest building scores for mice that were injected with a needle used 0 through 5 times were similar to those of mice injected with a needle had been used 16 through 20 times. Among the 37 reused needles that were tested, 4 were positive for bacterial growth; the only organisms cultured were *Staphylococcus* spp. Contrary to our hypothesis, reusing needles for subcutaneous injections did not increase animal stress based on analysis of vocalization or nest building.

Abbreviation: SBA, secondary bevel angle

DOI: 10.30802/AALAS-JAALAS-22-000094

Introduction

In the United States, the Occupational Safety and Health Administration regulates the use of hypodermic needles in human medicine to control the spread of bloodborne pathogens and minimize needlestick injuries.[6] In addition, the Centers for Disease Control prohibits using single-use needles on multiple patients[44] and has launched the One & Only campaign to eliminate unsafe medical injections in human healthcare.[7] However, the use of hypodermic needles for purposes involving animal blood or body fluids remains largely unregulated, or in some cases, is specifically excluded from federal and state provisions. Standard OSHA 1910.1030(b) defines the term 'blood' as human blood, human blood components, and products made from human blood.[6] Most states have drug paraphernalia laws that reference hypodermic needles.[30] However, this legislation does not extend to animal-related needle use. For example, New York state provisions overtly exclude livestock producers from limitations on the possession and dispensing of needles,[39] and the Illinois Hypodermic Syringes and Needles Act excludes persons engaged in chemical, clinical, pharmaceutical, or other scientific research, regardless of whether the person has medical training.[22]

A large body of literature is available on the use of hypodermic needles in human healthcare, covering such topics as vaccine development and distribution,[16,24,27] bloodborne pathogens,[8,15,38,43] addiction,[9,25,32,40] dentistry,[11,37] and diabetes.[34,42,52] Needle reuse in veterinary medicine has been evaluated in agriculture and food production[5,10,12,17,19,20,33] and in advanced procedures such as arthrocentesis,[1] laparoscopic surgery,[21,50] ultrasound-guided biopsy,[13] and advanced imaging.[35] However, we have been unable to find published scientific data regarding needle reuse in research animals.

Language describing needle reuse is largely absent from regulatory documents governing animal research. The USDA Animal and Plant Health Inspection Service Animal Welfare Act and Animal Welfare Regulations do not contain the word 'needle.'[3,4] The *Guide for the Care and Use of Laboratory Animals* mentions needles only once, identifying them as a potential physical safety hazard.[23] The *Guide for the Care and Use of Agricultural Animals in Research and Teaching* states that "Investigators and animal care staff should utilize best management practices associated with the use of syringes and handling needles" but does not elaborate on what those best practices are.[2] The *Biosafety in Microbiologic and Biomedical Laboratories* (BMBL) is slightly more descriptive about needle use, including language prohibiting recapping or removing a needle from a syringe, but only briefly mentions that an institution needs to have needle

Submitted: 28 Sep 2022. Revision requested: 02 Dec 2022. Accepted: 23 Jan 2023.
[1]Previously affiliated with Comparative Medicine, Worldwide Research, Development and Medical, Pfizer, Pearl River, New York; [2]Pharmaceutical Sciences Small Molecule Analytical Research and Development, Pfizer, Groton, Connecticut; and [3]Previously affiliated with Oncology Research and Development, Pfizer, Pearl River, New York
**Corresponding author. Email: ten32@cornell.edu*

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

policies consistent with applicable state, federal, and local requirements.[48] The Animal and Plant Health Inspection Service documents are descriptive rather than prescriptive, stating that blood collection and intramuscular injections in agricultural animals in a food production setting should be performed with a single-use needle.[47]

Many animal research institutions do not explicitly prohibit needle reuse.[28,49] However, welfare groups advocate using a one needle–one animal approach to minimize any risk to animal welfare.[31] Our institution (pharmaceutical research and development) recently implemented a global policy limiting needle reuse to a single cage of rodents (that is, 5 mice). An IACUC-approved oncology protocol at our institution contained an additional exemption that permitted reusing needles as many as 20 times in order to reduce the number of tumor cells lost to hub volume and the amount of time for filling syringes to maximize tumor cell viability.

The goal of the current study was to obtain quantifiable data on the effect of needle reuse on mice that received subcutaneous injections for the development of cell line xenograft and mouse allograft tumor models. To that end, digital imaging was used to measure secondary bevel angles of new and reused needles to determine the degree of needle blunting. Aerobic cultures of new and reused needles were performed to assess the potential for spread of pathogens between mice. Mouse vocalization and nest building were used to assess animal pain and distress. We hypothesized that data would reveal a little or no effect of needle reuse.

Materials and Methods

Animal subjects. This study was conducted at Pfizer (Pearl River, NY) when all authors were affiliated with that company. All study work was approved by the Pfizer Pearl River IACUC. All mice were housed in an AAALACi-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*.[23]

The study used 619 female mice (119 Crl:NU-*Foxn1^{nu}* and 500 BALB/cAnNCrl; 7 to 8 wks old; Charles River Laboratories, Wilmington, MA) that were housed in random groups of 5 in microisolation cages. At least 3 d were permitted for acclimation to use. Mouse groupings remained constant throughout the study. Room environmental parameters included controlled temperature (72 ± 2 °F [22 ± 1 °C]), relative humidity (50% ± 15%), and photoperiod (12:12-h light:dark; lights on, 0600). BALB/c mice were housed in reusable autoclaved individually ventilated polysulfone cages (GM500, Tecniplast, West Chester, PA). Nude mice were housed in disposable individually-ventilated polyethylene terephthalate caging (Innorack 3.5, Innovive, San Diego, CA). Both types of caging received 60 air changes per hour. All cages contained approximately 180 g of 1/4-in. (0.63-cm) corncob bedding (Bed-o'Cobs, The Andersons, Maumee, OH). All mice had free access to irradiated, low isoflavone, pelleted rodent chow (5V02, Purina Mills International, St. Louis, MO) and UV-sterilized, reverse osmosis–filtered municipal water chlorinated to 2 to 3 ppm. Cage enrichment included shredded paper bedding (Bed-r'Nests, The Andersons), a polycarbonate hut, and nylon chew bones (Bio-Serv, Flemington, NJ).

Based on vendor reports, BALB/c mice were free of mouse hepatitis virus, all mouse parvoviruses, mouse kidney parvovirus, ectromelia virus, K virus, polyoma virus, mouse cytomegalovirus, epizootic diarrhea of infant mice virus, mouse thymic virus, lactate dehydrogenase elevating virus, mouse norovirus, mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, Sendai virus, pneumonia virus of mice, respiratory enteric virus type 3, Hantaan virus, mouse adenovirus, *Mycoplasma pulmonis*, *Bordetella bronchiseptica*, *Streptococcus pneumoniae*, *Pasteurella* spp., *Salmonella* spp., *Streptobacillus moniliformis*, *Filobacter rodentium*, *Corynebacterium kutscheri*, *Helicobacter* spp., *Citrobacter rodentium*, *Clostridium piliforme*, *Pneumocystis murina*, *Encephalitozoon cuniculi*, major gastrointestinal metazoan endoparasites, major ectoparasites, and major enteric protozoa. Nude mice were also free of *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, β-hemolytic *Streptococcus*, *Corynebacterium bovis*, and *Proteus mirabilis*.

Injections. All needles used for injection were 25-gauge × 5/8-in. (1.6-cm), 1-mL slip tip needles (n = 359, Becton Dickinson, Franklin Lakes, NJ; n = 260, Nipro Medical, Miami, FL). Injections were made subcutaneously into the right flank (n = 414) or mammary fat pad (n = 205) by 3 staff members who had documented training and proficiency in performing such injections. Each mouse was injected with 0.2 mL of tissue culture cells in cell media. Each mouse was only injected once. After 5 mice were injected, the needle was gently removed, the syringe was refilled, and the needle was reattached onto the syringe. This process was repeated for a maximum of 4 times (20 mice) per needle. Needles that were dropped or seemed dull were discarded immediately. Data were collected from 5 cohorts of mice between 2019 and 2020.

Bacterial culture. Swabs of a subset of needles (n = 37; 8 nu/nu from cohort 1; 29 BALB/c [6 from cohort 2, 7 from cohort 3, 11 from cohort 4, and 5 from cohort 5]) were submitted for aerobic culture at a commercial laboratory (Charles River Laboratories, Wilmington, MA). After the last mouse was injected, the tip of the needle was gently wiped with a swab and placed into a culturette containing media (Amies gel without charcoal, BD BBL CultureSwab, Becton Dickinson). Among the cultured needles, 4 were controls (opened but never used for injection), 3 had been used 5 times, 1 had been used 10 times, 1 had been used 15 times, 2 had been used 19 times, and 26 had been used 20 times.

Needle imaging. After swabs had been obtained, needles were removed from syringes, placed into a 70% ethanol bath for 5 min, and then reattached to a 5-mL syringe containing 70% ethanol; the contents were expelled through the barrel of the used needle for disinfection. Needles were then carefully recapped, packaged, and shipped to another facility (Pfizer, Groton, CT) for imaging; unused needles were shipped to the imaging facility in their original manufacturer packaging. Extreme care was taken to ensure that needles did not contact any other surface during the disinfection and recapping process. Needles were inspected in room light and at 200× magnification by using a digital imaging system (VHX-6000, Keyence, Itasca, IL). Photomicrographs were captured using the Keyence Depth-up and Multilighting features. Plane measurement tools were applied to the images to quantify defects. The needles were viewed from the side, and the secondary bevel angle (SBA) was measured. The bevel angle and the bottom edge of the barrel are assumed to meet at a point, and the area between the needle and this point is equal to the area of deformation. These measures are consistent with the International Organization for Standardization.[24]

Nest building. Nest building was scored as previously described[1] for a subset of BALB/c mice (34 boxes, 170 mice total) from a single study cohort (cohort 4 of 5).[4] All housing enrichment materials present in the cage (huts, crinkled paper nesting material) were removed at time of injection. Then 10 ± 0.3 g of new Bed-r'Nest material (The Andersons) were added to each

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

box after injection of the last mouse in the box. The nest was scored 24 h later at approximately 7 to 9 h after room lights came on. When bedding material was untouched, the entire nest received a score of 0. When nest material had been manipulated but no distinct nesting site could be identified, the entire nest received a score of 1. When a distinct nesting site could be identified, the nest was analyzed as a square; each of the 4 corners received a score of 2 (nest was flat without shallow walls), 3 (wall height less than half the height of a dome), 4 (wall height equal to half the height of a dome), or 5 (wall taller than half the height of the dome). The 4 scores for each nest corner were then averaged to give an overall nest score. See reference 18 for a diagram.

Vocalization. All BALB/c mice (cohorts 2 through 5; $n = 500$) were evaluated for vocalization during injection. Mice that produced an audible noise at any point during injection or needle removal were considered to have vocalized; vocalizations that occurred before or after injection (i.e., during restraint only) were not counted. All mice were scored for vocalization by the same observer (TEB).

Statistical analyses. Statistical significance was defined as $p < .05$. Descriptive data were presented as mean values and standard deviations. Deformation from SBA was compared by using an unequal variances *t* test. Nest building scores were analyzed by using one-way ANOVA. Vocalization was analyzed by using a X^2 test. All analyses were performed by using SAS 9.4 (SAS Institute, Cary, NC). Figures were made by using Prism 8 (GraphPad Software, La Jolla, CA).

Results

Bacterial culture. Of the 37 needles cultured, 4 (11%) tested positive for bacterial growth on TBA agar. One needle used for 5 injections in cohort 4 tested positive for *Staphylococcus hominis*, one needle used for 15 injections in cohort 3 tested positive for *S. lentus*, and 2 needles used for 20 injections in cohort 3 tested positive for *S. nepalensis*. Bacteria were cultured only from needles that had been used to inject BALB/c mice.

Photomicrography. Representative images of unused needles ($n = 22$) and needles reused 20 times ($n = 23$) are shown in Figure 1. The SBA did not differ significantly between new ($M = 19.28°$) and reused ($M = 19.24°$) needles, $t(40.66) = 0.29$, $p = .77$ (Figure 1 B). The area of deformation relative to the SBA did not differ significantly between new ($M = 4577$ µm^2, $SD = 2349.7$) and reused ($M = 4178$ µm^2, $SD = 2193.3$) needles, $t(42.45) = 0.59$, $p = .56$ (Figure 1 C). The needle with the largest

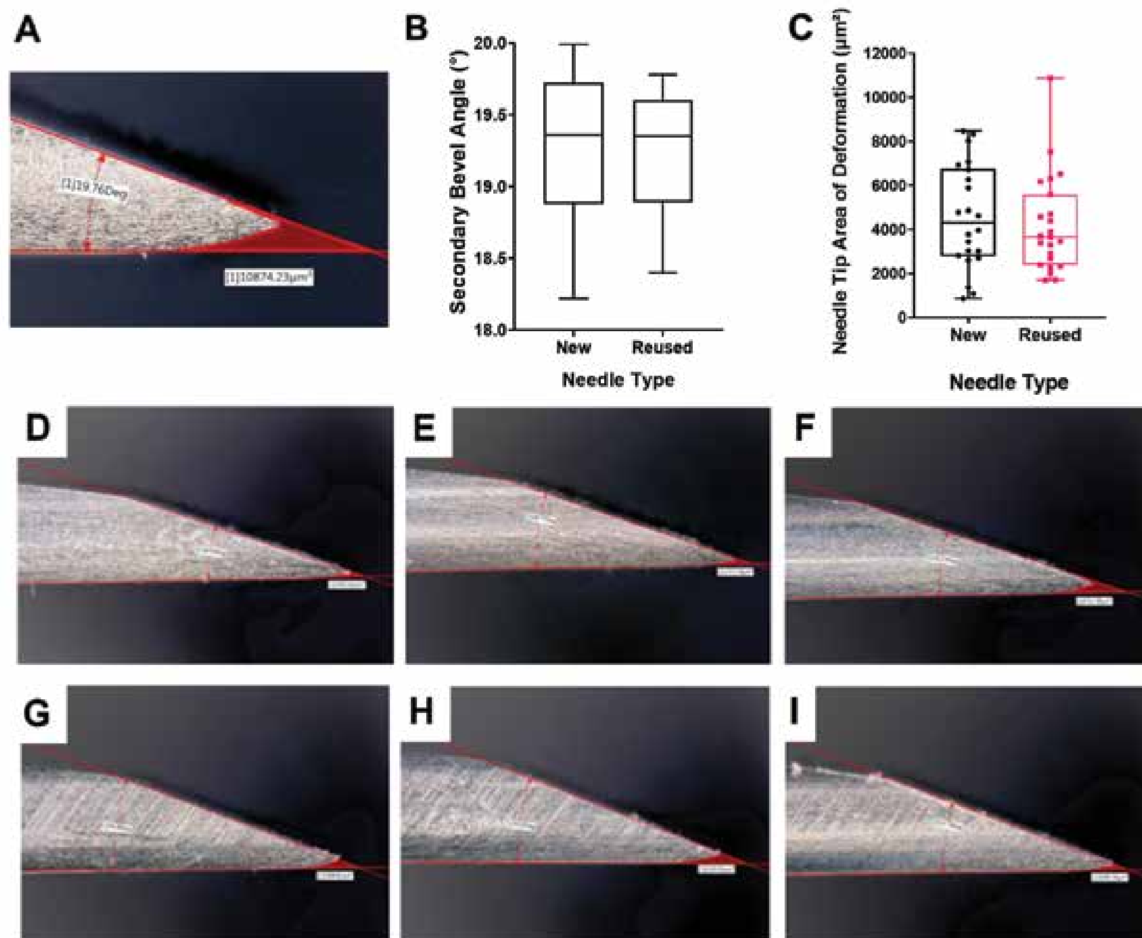

Figure 1. Measurement of the secondary bevel angle and area of deformation of new and reused hypodermic needles. Needles (25-gauge × 5/8-in., 1-mL slip tip) that were unused or reused for 20 subcutaneous injections in SPF mice were observed in room light at 200× magnification. Plane measurement tools of photomicroscopy software was used to calculate the secondary bevel angle (red straight lines); using the secondary bevel angle, the area of deformation was calculated by using the individual area function (shaded red area). (A) A needle that had been reused for 20 subcutaneous injections; this needle had the largest area of deformation among all needles imaged. Neither (B) secondary bevel angle nor (C) area of deformation differed between new and reused needles ($p = .77$ and $p = .56$, respectively). Included are representative images from (D–F) 3 unused needles and (G–I) 3 needles reused for 20 injections.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

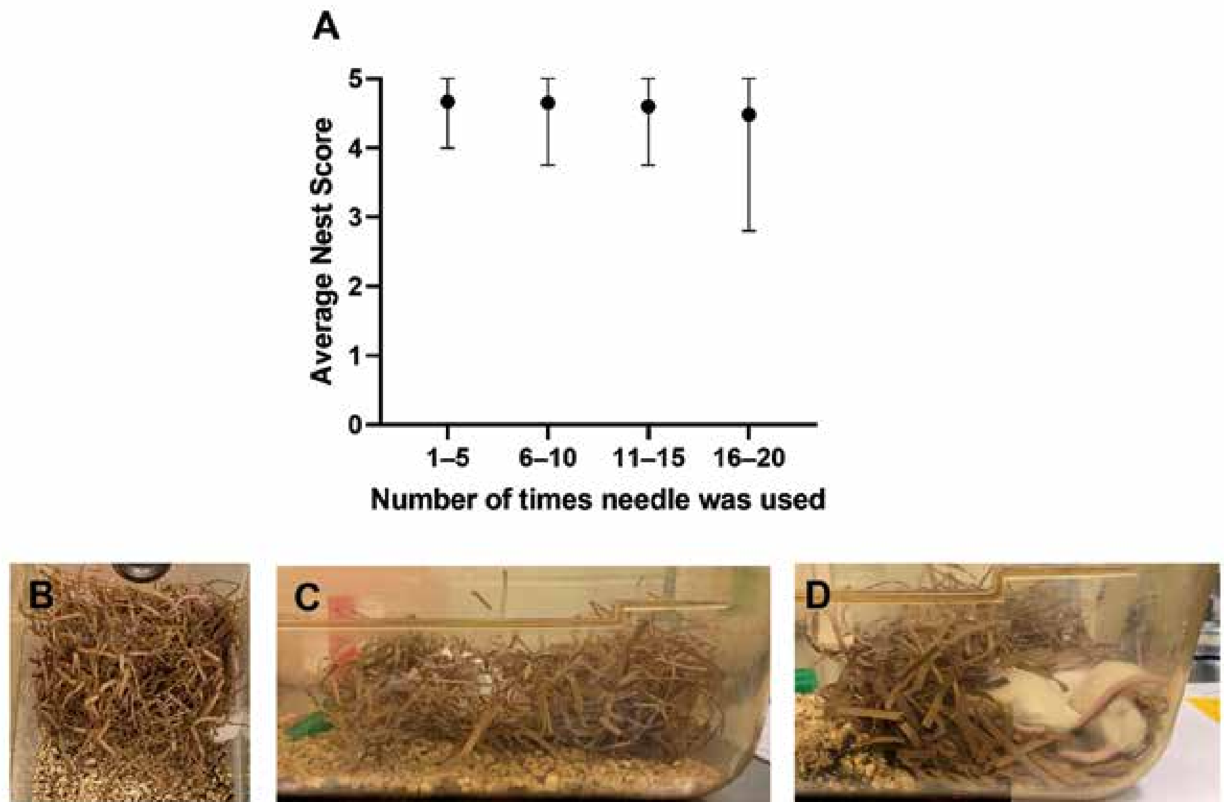

Figure 2. Nest building after injection with reused needles. A total of 255 BALB/c mice received subcutaneous injections with needles that had been reused for a maximum of 20 times. Mice were grouped by cage according to how many times the needle was used (1 through 5, 6 through 10, 11 through 15, or 16 through 20 times). At 24 h after injection, nests were scored according to the robustness of the nest that was created. (A) Average nest scores did not differ between groups ($p = .70$). (B) Aerial and (C) and side views of a nest that received a score of 5 for each corner. (D) A nest that received a score of 5 for the left corner and a 2 for the right corner.

area of deformation (10,874.23 μm^2) had been reused 20 times (Figure 1 A).

Nest building. Boxes of mice were categorized based on the number of times the needle had been reused for those mice at time of injection. Mean 24-h nest scores were 4.7 (1 through 5 times), 4.6 (6 through 10), 4.6 (11 through 15), and 5.48 (16 through 20). Nest scores did not differ significantly between groups, $F(3, 47) = 0.48$, $p = .70$ (Figure 2 A).

Vocalization. Mouse vocalization audible to humans during injection showed no significant relationship with the number of times a needle had been used, $X^2(19) = 23.98$, $P = .20$ (Figure 3).

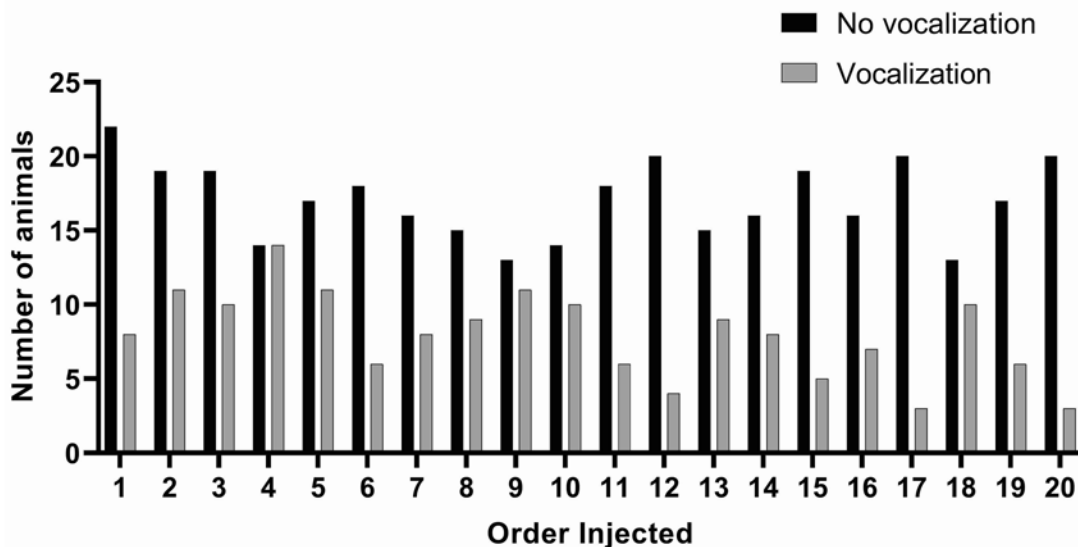

Figure 3. Vocalization after subcutaneous injection with reused needles. A total of 500 BALB/c mice were observed during subcutaneous injection of tissue culture cells in cell media for producing tumor models. Mice that produced an audible noise at any point during injection or needle removal were marked as having vocalized. Mouse vocalization showed no relationship to the number of times a needle was used ($p = .20$).

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

Discussion

Reusing a needle for multiple injections increases the risk of pain and infection in human and veterinary patients.[10,32,49] Our IACUC requested objective data regarding needle reuse in mice during the creation of cell line xenograft and mouse allograft tumor models. Oncology researchers held an exemption for needles to be reused 20 times, contrary to our standard institutional policy of 5 times. The justification was that tumor cells are collected in a specific volume and thus become a limited resource for inoculation into mice. Many cell lines are suspended in viscous media, which creates large air bubbles that must be voided. Increasing the time spent during the injection process theoretically threatens cell viability, potentially increasing the number of mice required for tumor modeling studies.

Veterinary staff at our institution initially explored switching to low hub-space needle-syringe combinations, but these needles could not be removed from the syringes, which was an important initial step for loading cell material into the syringe. We therefore evaluated methods and materials that were already in-use for the oncology studies in the current study.

The methods of pain assessment that we used here were chosen due to limited access to the mice. Creating a specific animal use protocol that includes additional measurements of stress was viewed as an unjustifiable use of animal resources. Imaging and swabbing the needles after use was a simple task that did not alter procedures currently approved on the protocol. Similarly, analysis of nest building and vocalization did not require animal manipulation and could be performed by a single observer, in contrast to the time to integrate to nest test (TINT)[36] or mouse grimace scoring.[29] Audible vocalization was analyzed instead of ultrasonic vocalization because published literature suggests that ultrasonic analysis may not be any more useful for analyzing acute, momentary pain than audible vocalizations.[51]

The AWA considers injection with a hypodermic needle the upper limit for a procedure in an animal before deeming it 'painful.'[3] However, mice are not a covered species under the AWA, and many approaches to assessing rodent pain and nociception may be unreliable for assessing in mild discomfort due to injection alone.

We considered other quantifiable methods of measurement, including skin histopathology and needle penetration performance, but study endpoints depended on tumor growth, and none of the mice required euthanasia immediately after injection, when histopathologic analysis would have been most valuable. In addition, statistical consultation suggested an extraordinarily large number of mice would be needed to observe acute histologic changes due to subcutaneous injection. Visual health checks were performed once daily as a part of our normal husbandry routine, and veterinary staff did not receive any reports of abnormal skin lesions after injection.

The International Organization for Standardization has developed and published international standards regarding single-use hypodermic needles.[24] As described in this standard, the SBA should be 17° ± 2°. The average SBA for both new and unused needles in the present study ($M = 19.28°$, $SD = 0.5$ and $M = 19.24°$, $SD = 0.4$ respectively) fell outside this range. The proportion of new needles that did not fall within the ISO standard was 15 of 22 (68%), as compared with 17 of 23 (74%) of reused needles. However, we chose not to exclude any needles from our dataset because the person administering the injection could not judge the SBA of an unused, manufacturer-packaged needle without having conducted imaging prior to the start of the study.

The only needles that tested positive for bacterial growth were those used on immunocompetent, SPF BALB/c mice. One needle tested positive for *S. hominis*, which is commonly isolated from human skin and may have an active role in healthy skin protection.[41] *S. nepalensis* and *S. lentus* have been isolated from the skin of SPF naïve C57BL/6 mice,[45] although the pathologic significance of these species is uncertain. *Staphylococcus* spp. are not excluded from immunocompetent mice at our facility or by the vendor. The skin of study mice, cell cultures or media, and the procedure area were not cultured, so we cannot draw conclusions about whether the isolated bacteria came from the skin of the mice or from another location. No bacteria were isolated from needles used on immunodeficient nu/nu mice, which were bred and maintained at the vendor at a health status that excluded additional specific opportunistic organisms, including *S. aureus*.

The scope of our current study is limited to subcutaneous injections for tumor creation in mice. Skin thickness is not consistent between species or even among different strains of mice,[46] and additional resistance from injecting through thicker skin might change the SBA and area of deformation after needle reuse. Other contributing factors could be the gauge of needle, the route of injection, or the material being injected.

In conclusion, reusing hypodermic needles for subcutaneous injection in SPF mice for as many as 20 times does not significantly increase needle defects or adversely affect nest building or animal vocalization. We do not recommend standardizing needle reuse but share our results to encourage institutions to challenge historical practices and make evidence-based decisions.

Acknowledgments

We thank Vlad Buklan, Christine Hosselet, LuAnna Lemon, John Liang, and Julita Ramirez.

References

1. **Adams SB, Moore GE, Elrashidy M, Mohamed A, Snyder PW.** 2010. Effect of needle size and type, reuse of needles, insertion speed, and removal of hair on contamination of joints with tissue debris and hair after arthrocentesis. Vet Surg **39**:667–673. https://doi.org/10.1111/j.1532-950X.2010.00649.x.
2. **American Society of Animal Science.** 2020. Guide for the care and use of agricultural animals in research and teaching, 4th ed. Champaign (IL): American Society of Animal Science.
3. Animal Welfare Act as Amended. 2008. 7 USC §2131–2156.
4. Animal Welfare Regulations. 2008. 9 CFR §2131–2156.
5. **Barbosa da Silva TI, Kohara Melchior LA, Santos PS, Reckziegel GH, Gomes YA, Carvalho Fernandes AC, da Silva DD, Baptista Filho LCF, Honório de Melo LE.** 2019. Risk factors for enzootic leukosis in dairy cattle from the Brazilian Amazon. Acta Sci Vet **47**:1683. https://doi.org/10.22456/1679-9216.96577.
6. **Bloodborne Pathogens as Amended.** 2019. 29 CFR § 1910:1030.
7. **Centers for Disease Control and Prevention.** [Internet]. 2019. One & only campaign. [Cited 26 September 2022]. Available at: https://www.cdc.gov/injectionsafety/one-and-only.html.
8. **Chung YS, Choi JY, Han MG, Park KR, Park SJ, Lee H, Jee Y, Kang C.** 2018. A large healthcare-associated outbreak of hepatitis C virus genotype 1a in a clinic in Korea. J Clin Virol **106**:53–57. https://doi.org/10.1016/j.jcv.2018.07.006.
9. **Clarke K, Harris D, Zweifler JA, Lasher M, Mortimer RB, Hughes S.** 2016. The significance of harm reduction as a social and health care intervention for injecting drug users: An exploratory study of a needle exchange program in Fresno, California. Soc Work Public Health **31**:398–407. https://doi.org/10.1080/19371918.2015.1137522.
10. **Darpel KE, Barber J, Hope A, Wilson AJ, Gubbins S, Henstock M, Frost L, Batten C, Veronesi E, Moffat K, Carpenter S, Oura C, Mellor PS, Mertens PPC.** 2016. Using shared needles for subcutaneous inoculation can transmit bluetongue

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

virus mechanically between ruminant hosts. Sci Rep **6**:20627. https://doi.org/10.1038/srep20627.

11. **Dau M, Buttchereit I, Ganz C, Frerich B, Anisimova EN, Daubländer M, Kämmerer PW.** 2017. Influence of needle bevel design on injection pain and needle deformation in dental local infiltration anesthesia - randomized clinical trial. Int J Oral Maxillofac Surg **46**:1484–1489. https://doi.org/10.1016/j.ijom.2017.06.013.
12. **De Almeida ÍC, Almeida SLH, Barioni G, Donatele DM, Clipes RC, Glória LS, Di Filippo PA.** 2021. Seroprevalence and influence of bovine leukemia virus on the incidence of mastitis in dairy herds. Acta Sci Vet **49**:1783. https://doi.org/10.22456/1679-9216.109458.
13. **de Rycke LJM, van Bree HJJ, Simoens PJM.** 1999. Ultrasound-guided tissue-core biopsy of liver, spleen and kidney in normal dogs. Vet Radiol Ultrasound **40**:294–299. https://doi.org/10.1111/j.1740-8261.1999.tb00364.x.
14. **Deacon RM.** 2006. Assessing nest building in mice. Nat Protoc **1**:1117–1119. https://doi.org/10.1038/nprot.2006.170.
15. **Deuffic-Burban S, Delarocque-Astagneau E, Abiteboul D, Bouvet E, Yazdanpanah Y.** 2011. Blood-borne viruses in health care workers: Prevention and management. J Clin Virol **52**:4–10. https://doi.org/10.1016/j.jcv.2011.05.016.
16. **Edens C, Collins ML, Ayers J, Rota PA, Prausnitz MR.** 2013. Measles vaccination using a microneedle patch. Vaccine **31**:3403–3409. https://doi.org/10.1016/j.vaccine.2012.09.062.
17. **Erskine RJ, Bartlett PC, Byrem TM, Render CL, Febvay C, Houseman JT.** 2012. Herd-level determinants of bovine leukaemia virus prevalence in dairy farms. J Dairy Res **79**:445–450. https://doi.org/10.1017/S0022029912000520.
18. **Gaskill BN, Karas AZ, Garner JP, Pritchett-Corning KR.** 2013. Nest building as an indicator of health and welfare in laboratory mice. J Vis Exp **82**:51012.
19. **Horner RF.** 1982. Malignant oedema caused by *Clostridium perfringens* type A in a horse. J S Afr Vet Assoc **53**:122–123.
20. **Hutchinson HC, Norby B, Erskine RJ, Sporer KRB, Bartlett PC.** 2020. Herd management practices associated with bovine leukemia virus incidence rate in Michigan dairy farms. Prev Vet Med **182**:105084. https://doi.org/10.1016/j.prevetmed.2020.105084.
21. **Hyink S, Whittemore JC, Mitchell A, Reed A.** 2013. Diagnostic accuracy of tissue impedance measurement interpretation for correct Veress needle placement in feline cadavers. Vet Surg **42**:623–628. https://doi.org/10.1111/j.1532-950X.2013.01098.x.
22. **Hypodermic Syringes and Needles Act as Amended.** 2019. 720 ILCS 635/2.
23. **Insitute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
24. **International Organization for Standardization.** [Internet]. 2016. Sterile hypodermic needles for single use — Requirements and test methods (Standard No. 7864). [Cited 28 September 2022. Available at: https://www.iso.org/standard/60481.html.
25. **Jia Y, McCluskie MJ, Zhang D, Monette R, Iqbal U, Moreno M, Sauvageau J, Williams D, Deschatelets L, Jakubek ZJ, Krishnan L.** 2018. In vitro evaluation of archaeosome vehicles for transdermal vaccine delivery. J Liposome Res **28**:305–314. https://doi.org/10.1080/08982104.2017.1376683.
26. **Jones CM.** 2018. Trends and key correlates of prescription opioid injection misuse in the United States. Addict Behav **78**:145–152. https://doi.org/10.1016/j.addbeh.2017.10.018.
27. **Kastenmuller W, Gasteiger G, Stross L, Busch DH, Drexler I.** 2009. Cutting edge: Mucosal application of a lyophilized viral vector vaccine confers systemic and protective immunity toward intracellular pathogens. J Immunol **182**:2573–2577. https://doi.org/10.4049/jimmunol.0803871.
28. **Kieffer J.** [Internet]. 2019. The Ohio State College of Food, Agricultural, and Environmental Sciences needle safety policy. [Cited 26 September 2022]. Available at: https://cfaessafety.osu.edu/sites/safety/files/imce/CFAES%20Needle%20Safety%20Policy%5B2%5D.pdf.
29. **Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS.** 2010. Coding of facial expressions of pain the laboratory mouse. Nat Methods **7**:447–449. https://doi.org/10.1038/nmeth.1455.
30. **Legislative Analysis and Public Policy Association.** [Internet]. 2022. Drug paraphernalia: Summary of state laws. [Cited 26 September 2022]. Available at: https://legislativeanalysis.org/wp-content/uploads/2022/04/Drug-Paraphernalia-Summary-of-State-Laws-FINAL-1.pdf.
31. **National Centre for the Replacement, Refinement, and Reduction of Animals in Research.** [Internet]. 2020. Single use of needles. [Cited 26 September 2022]. Available at: https://www.nc3rs.org.uk/3rs-resources/single-use-needles.
32. **Noroozi M, Armoon B, Ghisvand H, Noroozi A, Karimy M, Bazrafshan MR, Marshall BDL, Dieji B.** 2019. Prevalence and risk factors for injection site skin infections among people who inject drugs (PWID) in Tehran. J Cosmet Dermatol **18**:258–262. https://doi.org/10.1111/jocd.12675.
33. **Owen K, Blackie N, Gibson TJ.** 2022. The effect of needle reuse on piglet skin puncture force. Vet Sci **9**:90. https://doi.org/10.3390/vetsci9020090.
34. **Puder JJ, Atar M, Muller B, Pavan M, Keller U.** 2005. Using insulin pen needles up to five times does not affect needle tip shape nor increase pain intensity. Diabetes Res Clin Pract **67**:119–123. https://doi.org/10.1016/j.diabres.2004.06.001.
35. **Raval AN, Karmarkar PV, Guttman MA, Ozturk C, Desilva R, Aviles RJ, Wright VJ, Schenke WH, Atalar E, McVeigh ER, Lederman RJ.** 2006. Real-time MRI guided atrial septal puncture and balloon septostomy in swine. Catheter Cardiovasc Interv **67**:637–643. https://doi.org/10.1002/ccd.20579.
36. **Rock ML, Karas AZ, Gartrell Rodriguez KB, Gallo MS, Pritchett-Corning K, Karas RH, Aronovitz M, Gaskill BN.** 2014. The time-to-integrate-to-nest test as an indicator of wellbeing in laboratory mice. J Am Assoc Lab Anim Sci **53**:24–28.
37. **Rout PG, Saksena A, Fisher SE.** 2003. An investigation of the effect on 27-gauge needle tips following a single local anesthetic injection. Dent Update **30**:370–374. https://doi.org/10.12968/denu.2003.30.7.370.
38. **Saginur R, Nixon J, Devries B, Bruce N, Carruthers C, Scully L, Berger R, Leech J, Nicolle L, Mackenzie A.** 2001. Transmission of hepatitis C in a pharmacologic study. Infect Control Hosp Epidemiol **22**:697–700. https://doi.org/10.1086/501848.
39. **Sale and possession of hypodermic syringes and hypodermic needles.** 2021. NY Public Health (PBH) Ch 45, Article 33, Title 7.
40. **Sanchez DP, Tookes H, Pastar I, Lev-Tov H.** 2021. Wounds and skin and soft tissue infections in people who inject drugs and the utility of syringe service programs in their management. Adv Wound Care (New Rochelle) **10**:571–582. https://doi.org/10.1089/wound.2020.1243.
41. **Severn MM, Williams MR, Shahbandi A, Bunch ZL, Lyon LM, Nguyen A, Zaramela LS, Todd DA, Zengler K, Cech NB, Gallo RL, Horswill AR.** 2022. The ubiquitous human skin commensal *Staphylococcus hominis* protects against opportunistic pathogens. MBio **13**:e0093022. https://doi.org/10.1128/mbio.00930-22.
42. **Shen M, Shi Y, Zheng S, Fan H, Xu J, Yang T.** 2021. A systematic survey of physicians' insights into lipohypertrophy. Front Public Health **9**:738179. https://doi.org/10.3389/fpubh.2021.738179.
43. **Sherertz EF, Davis GL, Rice RW, Harris BA, Franzini DA.** 1986. Transfer of hepatitis B virus by contaminated reusable needle electrodes after electrodesiccation in simulated use. J Am Acad Dermatol **15**:1242–1246. https://doi.org/10.1016/S0190-9622(86)70297-1.
44. **Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee.** [Internet]. 2022. 2007 Guideline for isolation precautions: Preventing transmission of infectious agents in healthcare settings. [Cited 26 September 2022]. Available at: https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html.
45. **Tavakkol Z, Samuelson D, deLancey Pulcini E, Underwood RA, Usui ML, Costerton JW, James GA, Olerud JE, Fleckman P.** 2010. Resident bacterial flora in the skin of C57BL/6 mice housed under SPF conditions. J Am Assoc Lab Anim Sci **49**:588–591.
46. **Todo H.** 2017. Transdermal permeation of drugs in various animal species. Pharmaceutics **9**:33.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

47. **United States Department of Agriculture Animal and Plant Health Inspection Service.** [Internet]. 2021. Bovine leukemia virus (BLV). [Cited 28 September 2022]. Available at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/cattle-disease-information/bovine-leukemia-disease-info/index.
48. **United States Department of Health and Human Services.** 2020. Biosafety in microbiological and biomedical laboratories, 6th ed. Bethesda (MD): National Institutes of Health.
49. **Welter J, Patten C Jr.** [Internet]. University of Wisconsin-Madison, research animal resources and compliance, the reuse of needles in research animals. [Cited 26 September 2022]. Available at: https://www.rarc.wisc.edu/policies/aur_2_the_reuse_of_needles.html.
50. **Whittemore JC, Mitchell A, Hyink S, Reed A.** 2013. Diagnostic accuracy of tissue impedance measurement interpretation for correct Veress needle placement in canine cadavers. Vet Surg **42**:613–622. https://doi.org/10.1111/j.1532-950X.2013.01107.x.
51. **Williams WO, Riskin DK, Mott KM.** 2008. Ultrasonic sound as an indicator of acute pain in laboratory mice. J Am Assoc Anim Sci **47**:8–10.
52. **Zabaleta-del-Olmo E, Vlacho B, Jodar-Fernández L, Urpí-Fernández AM, Lumillo-Gutiérrez I, Agudo-Ugena J, Morros-Pedrós R, Violán C.** 2016. Safety of the reuse of needles for subcutaneous insulin injection: A systematic review and meta-analysis. Int J Nurs Stud **60**:121–132. https://doi.org/10.1016/j.ijnurstu.2016.04.010.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25