Animal Welfare in High Containment or Barrier Facilities: Addressing Unique Challenges

NIH National Institutes of Health
Office of Laboratory Animal Welfare

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Photo credit: CDC/James Gathany

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OLAW Online Seminar Series
June 9, 2022

Learning Objectives

- Understand the unique welfare challenges in high containment or barrier facilities
- Evaluate and implement methods to overcome those challenges
- Possess resources for future reference in preventing and addressing welfare challenges

Let Us Get to Know You…Polling Questions

Overview of Research Laboratories

Biosafety levels (BSL) are used to identify the protective measures needed in a laboratory setting to protect workers, the environment, and the public.

- **BSL-1:** infectious agents or toxins not known to consistently cause disease in healthy adults
- **BSL-2:** moderate-risk infectious agents or toxins that pose a risk if accidentally inhaled, swallowed, or exposed to the skin
- **BSL-3:** infectious agents or toxins that may be transmitted through the air and cause potentially lethal infection through inhalation exposure
- **BSL-4:** infectious agents or toxins that pose a high risk of aerosol-transmitted laboratory infections and life-threatening disease for which no vaccine or therapy is available

Biosafety level designations in the BMBL outline specific practices and safety and facility requirements.

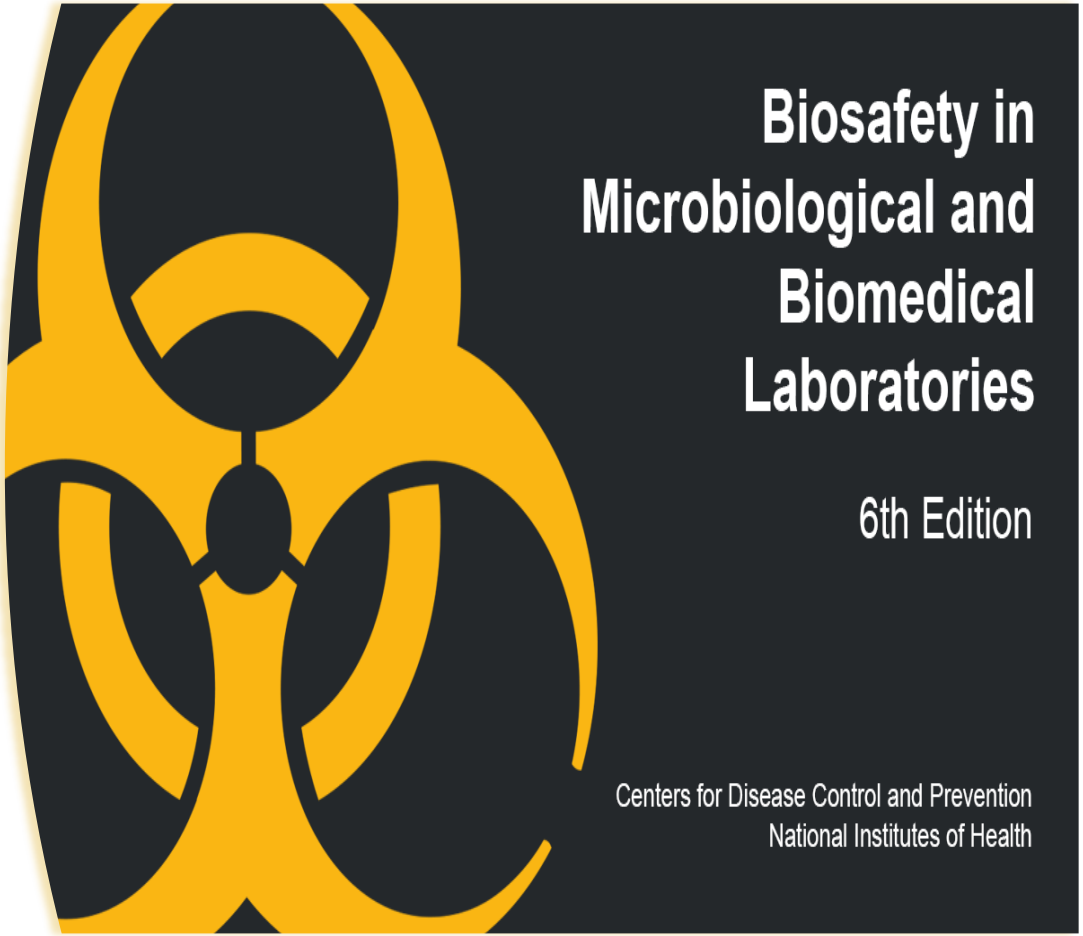

Table 1. Summary of Laboratory Biosafety Levels (BSLs)

BSL	Agents	Special Practices	Primary Barrier and Personal Protective Equipment	Facilities (Secondary Barriers)
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	• Access limited to those with need to enter; • Viable material removed from laboratory in primary and secondary containers, opened only in BSL-3 or ABSL-3 laboratories; • All procedures with infectious materials performed in a BSC	• BSCs for all procedures with viable agents; • Solid front gowns, scrubs, or coveralls; • Two pairs of gloves, when appropriate; • Protective eyewear; • Respiratory protection, as needed	• Physical separation from access corridors; • Access through two consecutive self-closing doors; • Hands-free sink near exit; • Windows are sealed; • Ducted air ventilation system with negative airflow into laboratory; • Autoclave available; preferably in laboratory
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	• Clothing change before entry; • Daily inspections of essential containment and life support systems; • All wastes decontaminated prior to removal from laboratory; • Shower on exit	• BSCs for all procedures with viable agents; • Solid front gowns, scrubs, or coveralls; [a] • Gloves; [b] • Full-body, air-supplied, positive-pressure suits[c]	• Entry sequence; • Entry through airlock with airtight doors, [c] walls, floors, ceilings form sealed internal shell; • Dedicated, non-recirculating ventilation system required; • Double-door, pass-through autoclave required

[a] Each successive BSL contains the recommendations of the preceding level(s) and the criteria in the cell.
[b] Applies to Cabinet Laboratory
[c] Applies to Suit Laboratory

HCL Entry and Egress

Photo credit: CDC/James Gathany

Photo credit: CDC/Dr. Scott Smith

HCL Entry and Egress

Photo credit: CDC/James Gathany

HCL Entry and Egress

High Containment and Barrier Facilities

- **High containment** **Keep organisms in**
- **Barrier** **Keep organisms out**
  - Sterile caging
  - Sterilization of supplies prior to use
  - Sterilized feed
  - Air-shower entry
  - Facility dedicated scrubs
  - Mask

Quarantine

Figure 1. Typical personal protective equipment worn in a BSL3 facility, including powered air-purifying respirator, Tyvek suit, solid-front gown, double gloves, and shoe covers.

Preclinical coronavirus studies and pathology: Challenges of the high-containment laboratory
https://journals.sagepub.com/doi/pdf/10.1177/03009858221087634

Welfare Challenges

Working in HCL/Barrier Facilities

- Entry/exit procedures
- Space limitations
- Decontamination
- Occupational health and safety considerations
  - Handling
  - Sample collection
  - Tools
- Study design
- Training

Photo credit: CDC/Nadia Gallardo, DVM

Entry/exit procedures

- Time and resource intensive
- Minimize the number of entries and the number of personnel present
- Health checks

Space Limitations

- Caging
- Storage

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Occupational Health and Safety Considerations

- Handling
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Study Design

Table 2 Scores for the different environments at PHE.

		Containment Level 2		Containment Level 3	
	Breeding colony	**CL2v1: Older style with upper extension**	**CL2v2: New style with balcony**	**CL3v1: High containment**	**CL3v2: High containment**
Housing	2	5	3	6/7/8*	4
Group size	1	3	¾	3/4/6	3
3D enrichment	1	4	3	6/7/8*	5
Manipulable enrichment	1	6	2	7	6
Average score	1.25	4.50	2.75/3.00	5.50/6.25/7.25	4.50

Each parameter scored between 1-10, where a score of 1 indicates the best possible state while a score of 10 would be the worst possible state.
* Scores depend on the weight of the animal: Lowest score < 4 kg. Intermediate score 4-6 kg. highest score > 6 kg.

Refinement of welfare through development of a quantitative system for assessment of lifetime experience
https://www.ingentaconnect.com/content/ufaw/aw/2015/00000024/00000002/art00002

Study Design

A

B

Figure 1 (A) Examples of toys and structures for climbing, resting, and hiding for group-housed rhesus macaques (photo courtesy of the Oregon National Primate Research Center). (B) Example of a hammock for a rhesus macaque (photo courtesy of the University of Massachusetts, Amherst).

Environmental enrichment in the 21st century
https://academic.oup.com/ilarjournal/article/58/2/295/3746523

Study Design

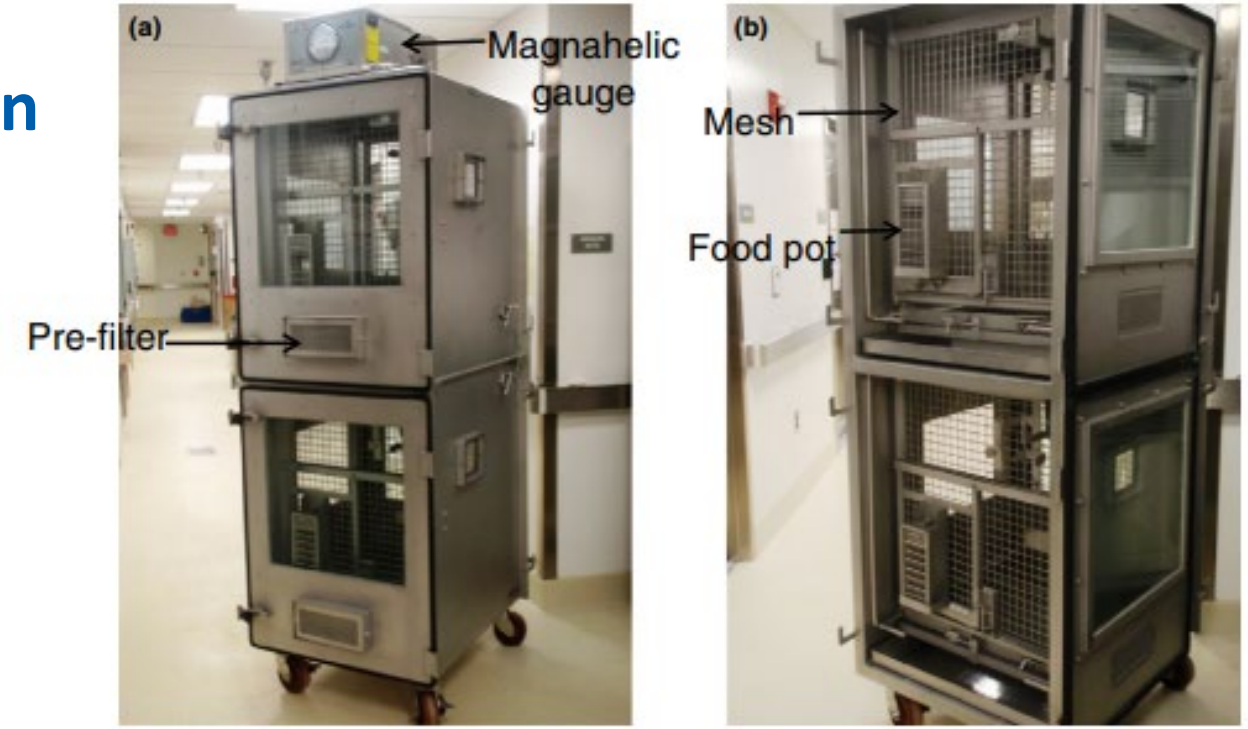


Fig. 2 Primate biocontainment caging. (a) Custom-designed NHP BCU caging in a one-over-one closed configuration. The cage is made of stainless steel with glass windows. Magnetic gauge is shown at the top. A prefilter is located on the lower front of each cage. HEPA exhaust filter is on the rear of the cage (not shown). (b) Primate BCU with front door open to reveal mesh cage front.

Novel adaptations to house marmosets at ABSL-3
https://academic.oup.com/femspd/article/71/2/219/2911570

Study Design

- Socially housed
- Complex environmental enrichment
  - Sleeping hammocks
  - Manipulanda
  - Pool
  - Multiple levels with feeding stations

Photo credit: CDC/Cassandra M. Tansey, DVM, DACLAM

Study Desig

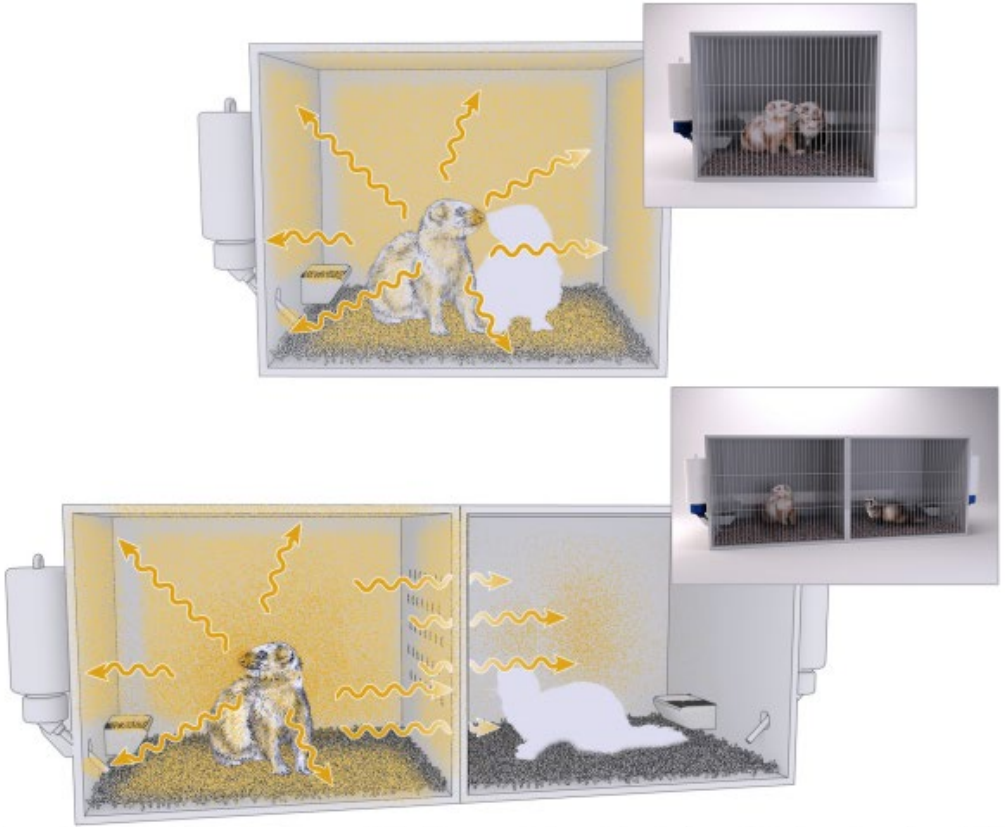
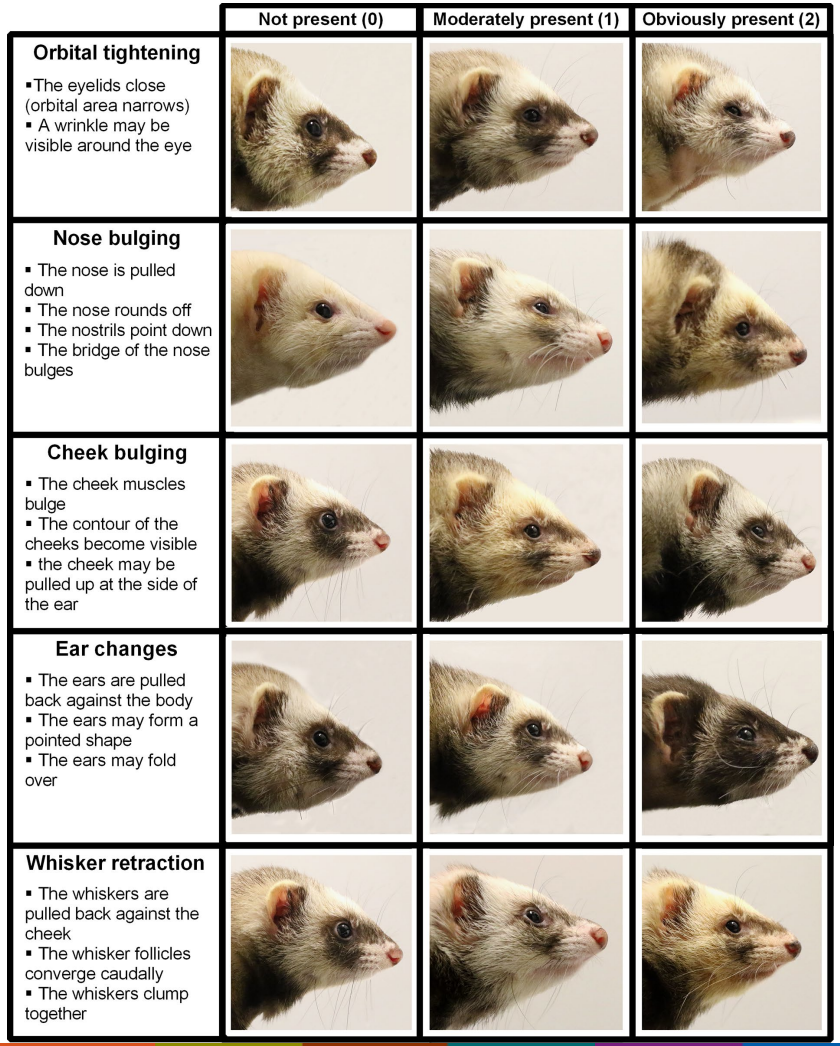


FIG 4 Differences in exposure to influenza virus between established ferret transmission models. Naive ferrets (white silhouette) are cohoused with inoculated ferrets (top, direct contact model) or placed adjacent to inoculated ferrets (bottom, respiratory droplet model). Areas of potential exposure to influenza virus are depicted in yellow. Arrows indicate dispersion of respiratory droplets expelled from the inoculated ferret.

Complexities in Ferret Influenza Virus Pathogenesis and Transmission Models
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4981671/

Training

- IACUC protocol review
- Animal welfare evaluations
- Humane endpoints
- Facility inspection



The composition and initial evaluation of a grimace scale in ferrets after surgical implantation of a telemetry probe
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0187986

Overcoming Challenges

Evaluating Animal Welfare

- Team approach
- Appropriate welfare indicators
- Interobserver reliability
- System for recording indicators

Table 3 Components of an "ideal" welfare state and examples of indicators associated with them.

Component	Characteristics	Examples of indicators
(i) Physical state	Good levels of physiological fitness, with no physical disabilities that either cause discomfort or pain, or that have an impact on physical function that could cause distress.	Indicators relating to the observable physical condition of the animals, e.g., body weight, state of the coat, posture, lameness and excessive attention to surgical sites
(ii) Physiological/ biochemical state	Levels of stress and distress do not exceed those that would occur during the course of normal social interactions, for example. If parameters such as heart rate or blood pressure were to be measured, they would not be expected to indicate significant stress.	Physiological parameters such as heart rate, respiratory rate, levels of stress hormones such as corticosteroids.
(iii) Psychological state	The animal displays an 'appropriate' range of behaviours, according to what is known about the species and strain.	Changes in behaviour such as increase aggression to cage mates, withdrawal, stereotypies and changes in use of enrichment.

A guide to defining and implementing protocols for the welfare assessment of laboratory animals
https://journals.sagepub.com/doi/pdf/10.1258/la.2010.010031

Evaluating Animal Welfare

- Team approach
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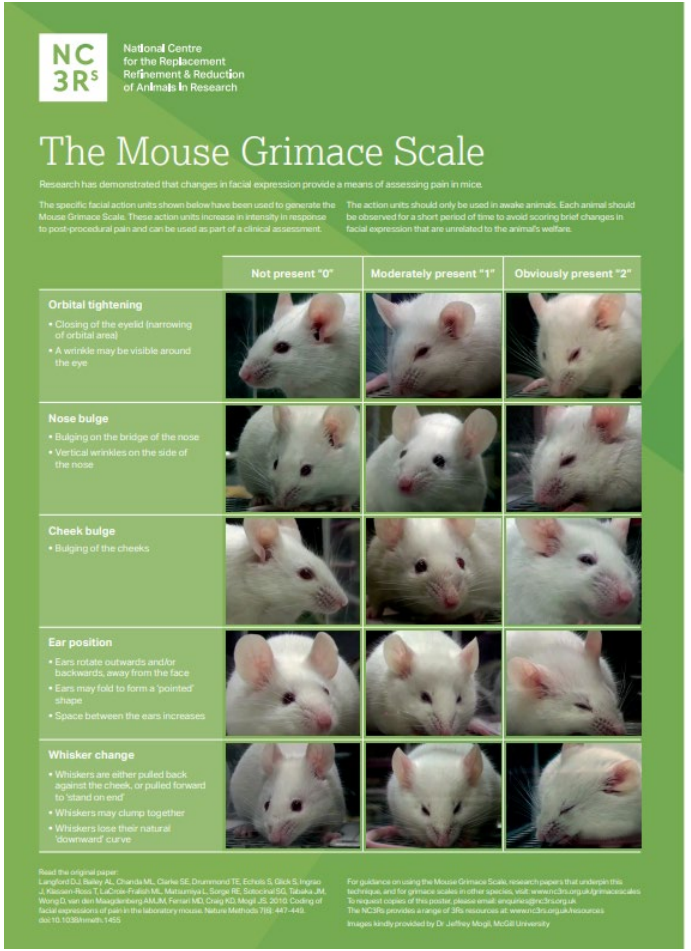
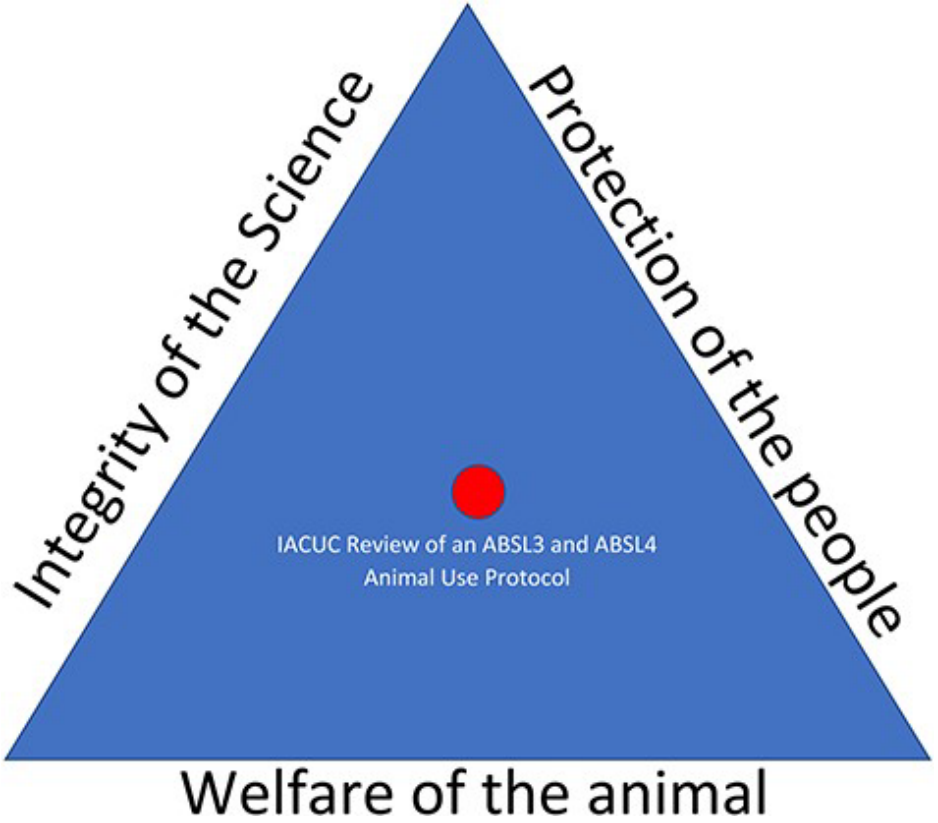
- Interobserver reliability
- System for recording indicators



Grimace scales
https://www.nc3rs.org.uk/3rs-resources/grimace-scales

Protocol Review

- Clinical observations
- Supportive care
- Scientific endpoints
- Euthanasia criteria
- Animal manipulations
- Documentation
- Personnel training
- Contingency plans
- Security
- Decontamination



IACUC and veterinary considerations for review of ABSL3 and ABSL4 research protocols
https://academic.oup.com/ilarjournal/article/61/1/3/6199911

Clinical Scoring

- Develop clinical scoring system
  - Identify critical clinical parameters
  - Species and agent specific
    - General appearance
    - Body condition score
    - Natural behavior
    - Responsiveness
- A single clinical sign may identify an endpoint

[A guide to defining and implementing protocols for the welfare assessment of laboratory animals](https://journals.sagepub.com/doi/pdf/10.1258/la.2010.010031)
https://journals.sagepub.com/doi/pdf/10.1258/la.2010.010031

Table 5 Identifying indicators for each adverse effect

What might the observable or measurable indicators be in an animal experiencing this effect? How should they be described?

How frequently should animals be monitored, and at what times, to ensure that the indicators will be picked up?

How could the indicators be assessed and which method is preferable and most feasible?

- Measured objectively?
- Observed and marked as present or absent?
- Assigned a numerical score?

Will the benefits of monitoring outweigh any disturbance that may be cause? Or, could disturbance be minimized by including welfare assessment when the animals will be disturbed anyway, e.g., at a project-driven body weight check?

Will invasive techniques be involved, such as blood sampling or implanting telemetry devices solely for monitoring purposes?*

Will measuring the indicators adversely affect the scientific outcome? Or conversely, could data gathered for scientific purposes also be used to assess welfare?

Can any environmental indicators be used, e.g., interaction with enrichment items such as climbing resources or nesting materials?

*The use of invasive procedures for welfare monitoring or implementing humane endpoints require a carefully considered harm-benefit assessment and consultation with veterinarians and regulators (the latter with respect to any legal implications). It may also affect the harm-benefit assessment of the project as a whole.

Clinical Scoring

A Guide for the Use of the Ferret Model for Influenza Virus Infection
https://www.sciencedirect.com/science/article/pii/S0002944019307692

Table 1 Detection and Reporting of Clinical Signs of Influenza Virus Infection in Ferrets

Clinical sign	Collection of data	Reporting of data	Anticipated detection*
Fever	Daily via s.c. transponder, telemetry, or rectal thermometer	Increase in °C above preinoculation baseline, reported as peak increase or graph of daily observations	LV: low and transient increase early after infection (e.g., days 1-3 p.i.) HV: elevated and sustained several days
Weight loss ‡	Daily	Percentage of weight reduction from preinoculation body weight, reported as peak weight loss or graph of daily observations	LV: mild to moderate weight loss (typically <15%) HV: moderate to severe weight loss (up to 25%)
Lethargy ‡	Daily visual assessment, video capture, telemetry, or objective measurement (e.g., treadmill	Relative inactivity index, quantification of videography movement, other scoring assessments	LV: none to mild lethargy during acute phase HV: sustained reduced activity
Nasal discharge/ rhinorrhea	Direct observation	Absence/presence	LV and HV: possible indicator of secondary bacterial infections
Diarrhea	Direct observation of perianal area or in bedding	Absence/presence	More frequent with HV or 2009 H1N1 pandemic virus, possible indicator of virus replication in gastrointestinal tissue
Ocular discharge/ complications	Direct observation of discharge or squinting	Absence/presence	Both LV and HV: possible indicator of secondary bacterial infection or virus replication in ocular tissue
Sneezing	Direct observation, video capture, or telemetry	Absence/presence	More frequently detected among viruses with increased transmissibility between ferrets
Anorexia/ inappetence	Monitored food intake concurrent with weight loss	Absence/presence	More frequently associated with HV
Dyspnea	Direct observation, video capture, or telemetry	Absence/presence	More frequently associated with HV
Alopecia	Direct observation	Absence/presence	More frequently associated with HV, where weight loss is concurrently detected.
Neurologic signs ‡	Torticollis, hind-limb weakness/ paralysis, extreme deviation from reinoculation disposition and/or aggression levels	Absence/presence	More frequently associated with HV, possible indicator of virus replication in brain tissue

*References supporting this information are found in the text.
‡ These parameters are frequently used in criteria fro humane euthanasia because of development of severe disease.
LV, viruses possessing low virulence in the ferret mode: HV, viruses possessing high virulence in the ferret model: p.i. after infection.

Euthanasia Criteria

All animals will be monitored daily by an experienced animal care technician, research staff, or the PI for signs of generalized clinical illness, including but not limited to: lameness, ruffled fur, nasal discharge, respiratory distress (changes in respiratory rate or effort), inappetence, hunched posture, changes in stool quality/quantity, lethargy, neurologic signs (ataxia, tremors, paresis, vestibular signs, or paralysis) and weight loss (animals are weighed a minimum of once weekly; baseline values are obtained on day of challenge, or up to 3 days prior).

A pain/euthanasia scale that takes into account the total health parameters of each individual animal will be utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Animals scored at 8-9 points will be monitored two times per day by trained animal care technicians, research staff, study PI, and/or veterinary staff. Animals scored at 10 total points or above will be humanely euthanized.

2 points each

- Quiet, dull, but responsive (QDR)
- Hunched/ruffled coat/piloerection
- Mild respiratory signs
- Mild neurological signs

3 points each

- Dehydration (eye recession)

5 points each

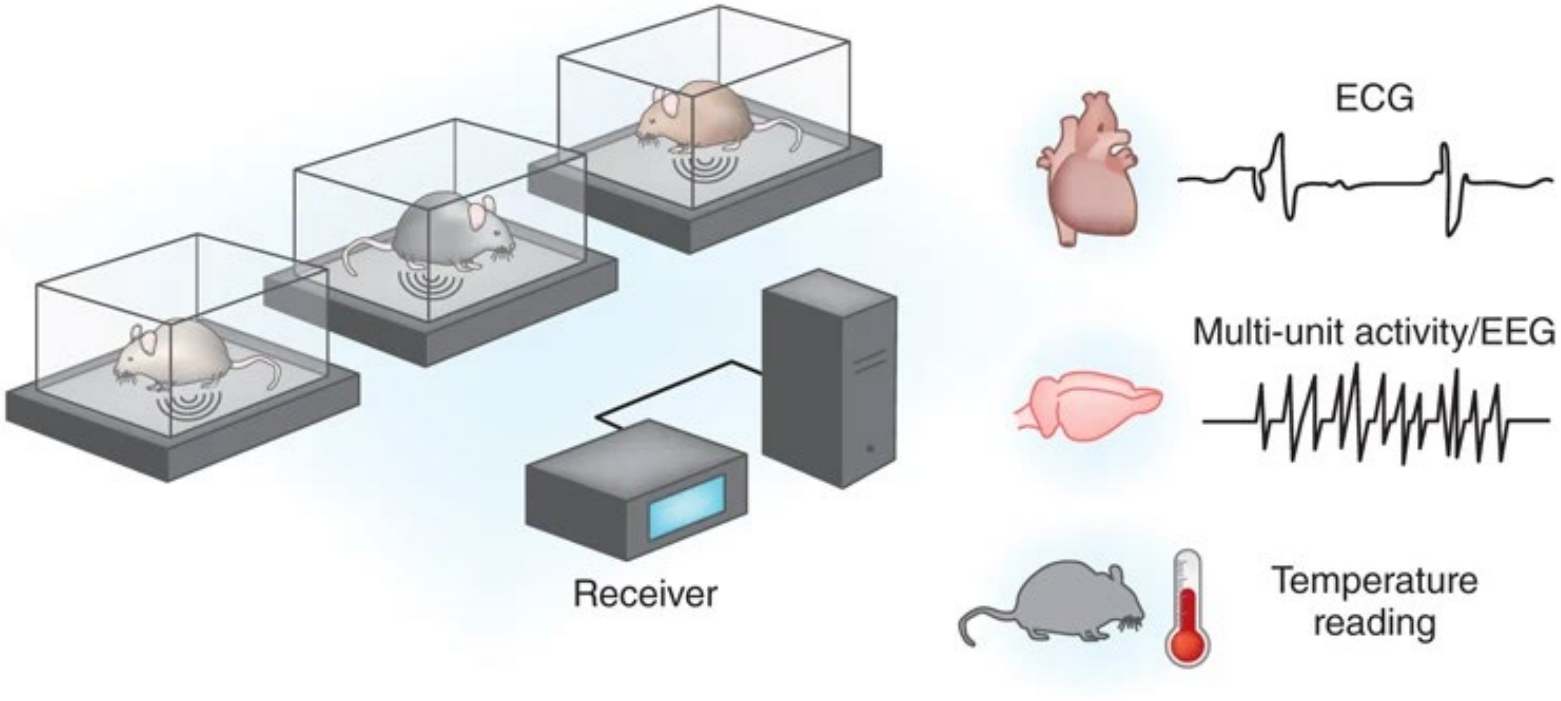
- Moderate respiratory signs (dyspnea, tachypnea)
- Moderate neurological signs (ataxia/circling/tremors/weakness)
- Hypothermia

10 points each

- Severe respiratory signs
- Severe neurological signs (paralysis)
- Frank hemorrhage
- Moribund
- >25% weight loss from baseline
- Unrelated trauma where continued exposure to cagemates is likely to lead to death or other immediate veterinary concerns as deemed by the veterinarian or Principal Investigator

Minimally Invasive Monitoring

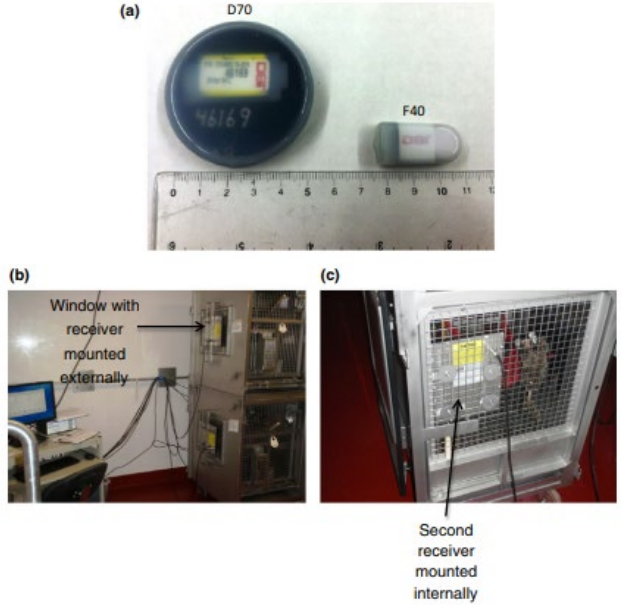
- Can
- Tele
- Cag



Telemetry for small animal physiology
https://www.nature.com/articles/laban.1048.pdf

Minimally Invasive Monitoring

- Cameras
- Telemetry
- Cage monitoring



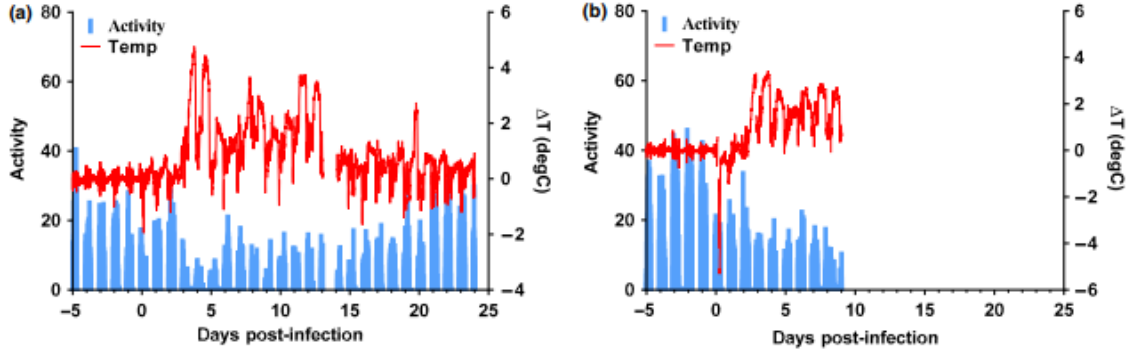


Fig. 5 Examples of telemetry data obtained from marmosets. Two receivers were mounted on each cage as described in the text. Both marmosets were infected with RVFV, and temperature and activity data were recorded at 15-min intervals for each animal. (a) A Marmoset that survived for the duration of the study, and (b) A marmoset that was euthanized due to severe illness 9 days after infection.

Novel adaptations to house marmosets at ABSL3
https://academic.oup.com/femspd/article/71/2/219/2911570

Animal Selection Considerations

- Medical history
- Temperament
- Social compatibility/group interactions
- Enrichment plans
- Training

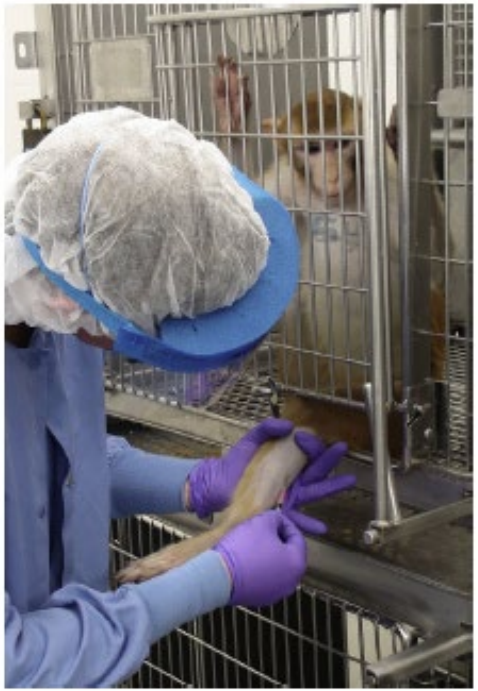


Figure 13.7 A juvenile rhesus monkey providing a voluntary venous blood sample.

Animal Selection Considerations

- Caging Requirements
  - Regulations
  - Observation
  - Ease of sanitation
  - Squeeze mechanism
  - Alarms
- Enrichment
  - Disposable
  - Autoclavable
  - Surface decontamination

Monkey Shine Mirrors
https://www.bio-serv.com/product/MSM.html

Hide N' Seek Shelter, Certified
https://www.bio-serv.com/product/HNS.html

Additional Resources

- Established in 2016
- Steering committee and working groups are organized under the following pillars:
  - Training world-class personnel
  - Scientific excellence
  - Institutional cooperation
  - International response

Biosafety Level 4 Zoonotic Laboratory Network
https://bit.ly/38X9sBT

National Centre for the Replacement Refinement & Reduction of Animals in Research

- Established in 2004
- Mission:
  - Support the discovery and adoption of predictive, reproducible and cost-effective alternatives to the use of animals.
  - Improve standards where animal use is necessary, optimising model selection and study design and minimising suffering as far as possible.
  - Promote the importance of the 3Rs across the scientific community, nationally and internationally, by providing training and embedding the 3Rs in policy, practice and regulations.

3Rs resource library
https://www.nc3rs.org.uk/3rs-resources

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Questions?

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Next Webinar: Fall 2022
Topic TBD

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