Tips and Tricks for the Care of NSG Mice

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Technical Information Services
The Jackson Laboratory’s Mission

Performing Research
Investigating genetics and biology of human disease

Providing Resources
JAX® Mice Clinical & Research Services, bioinformatics data, technical publications and more…

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World-class courses, internships and other programs

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The Gold Standard for Biomedical Research

- NIH funded resource
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- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
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  www.jax.org/jaxmice

- Mouse Genome Informatics
  www.informatics.jax.org

- Mouse Phenome Database
  www.jax.org/phenome

- And many more unique resources

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Presentation Overview

Handling and Care of the NSG Mouse
Presentation Overview

Learning Goals

- Explain features and uses of NSG mice
- Identify the types and signs of common infections in NSG mice
- Describe the processes and caging recommendations for successful care of NSG mice
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Immune System Components

Immunity

Innate

Adaptive

Cytokines

Complement system

Macrophages

Granulocytes

Natural killer (NK) cells

B lymphocytes

T lymphocytes

Antibodies

Dendritic cells
A Spectrum of Immune Deficiency

http://jinavie.tumblr.com/post/23052923021/three-little-pigs#.URU70WdL35w

Immune deficient

Immune competent

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Severely Immune Deficient Strains
“Fragile Superheroes”

- Nude
- Rag1/Rag2
- scid
- NOD scid gamma (NSG)
Immune System Components

**IMMUNITY**

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Immune System Components

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**Cytokines**

**B6 Rag1**
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NOD scid gamma
NSG, NOD scid gamma: Features

Official nomenclature: NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (005557)

Many Immunological Deficiencies

- NOD background contributes innate immune deficiencies
  - Macrophages, dendritic cells defective
  - No complement system
  - Bone marrow readily colonized by human hematopoietic stem cells
- scid mutation prevents development of mature T and B cells
- Il2rg gene knockout blocks signaling from 6 distinct interleukins and blocks NK cell development

Benefits:

- No scid leakiness
- Longer lifespan than NOD scid (mean lifespan ~22 months)
- Highly resistant to thymic lymphoma development, as compared to other scid mutant strains

To find NSG information online, visit www.jax.org/jaxmice/research/immunology/005557
NSG Research Applications
An incredibly useful and versatile immunodeficient mouse model

Research applications:
- Primary tumor engraftment
- Human hematopoiesis
- Humanized mice
- Infectious disease
- Regenerative medicine

To find NSG information online, visit www.jax.org/jaxmice/research/immunology/005557
Presentation Overview

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NSG mice
Infectious Disease Concerns

High level of immunodeficiency results in extreme susceptibility to:

- **Pathogenic microorganisms:**
  - Infectious agents that typically cause disease in immunocompetent hosts

- **Opportunistic microorganisms:**
  - Potentially infectious agents that rarely cause disease in immunocompetent hosts

- **Commensal microorganisms:**
  - Potentially infectious agents that reside in normal host tissues without causing disease

Pathogenic and Commensal Microbes

Common Threats to NSG mice

- C. bovis
- Citrobacter
- Enterobacter
- Enterococcus spp.
- Klebsiella spp.
- Proteus
- Pneumocystis murina
- Pseudomonas
- S. aureus
- Coagulase-negative Staphylococcus spp.
Infectious Disease Concerns
Opportunistic Infections

- Opportunistic microorganisms normally present in gut flora of healthy mice can become pathogenic in NSG mice, most commonly:
  - Klebsiella oxytoca
  - Enterococcus spp.

- Clinical Signs:
  - Hunched posture, scruffy coat
  - Females (twice as likely to be infected)

Contributing Factors to Mortality
Opportunistic Infections

- Urinary tract infections (UTIs)
  - Normal intestinal flora
  - Estrogen supplementation (increased risk)
- Ascending renal infection and pathology
- Secondary infection after primary insult
  - Skin wounds (ie needle punctures, bacterial dermatitis)
  - Molar gingival sulcus
- Breeding (often lactating) females more susceptible
Signs of Infection
Commonly Observed in NSG mice

- Infected skin wounds, cellulitis
- Abscesses (skin and internal organs)
- Otitis media, conjunctivitis, panophthalmitis
- Localized and widespread infections involving liver, heart, lungs, uterus, accessory sex glands, etc.

Infections in NSG mice

Contributing Factors

- Can occur in mice housed under less strict barrier conditions, strict barrier conditions, and isolators
- Most often seen after mice have been in the facility a while
- Often appear to be an individual animal vs. colony issue
  - Sporadic
  - Sick and healthy (or infected/non-infected) animals commonly in the same cage
Presentation Overview

Learning Goals

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- Describe husbandry and caging recommendations for successful care of NSG mice
How Clean is Clean Enough?

Decontamination

Sanitization:
- Reduction of microbial organisms from inanimate surfaces

Disinfection:
- Destruction or inactivation of most microbial organisms from inanimate surfaces

Sterilization:
- Complete destruction or inactivation of all microbial organisms from inanimate surfaces

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How Clean is Clean Enough? Housing Conditions

- Barrier practices adequate to maintain nude, or even scid mice may not be adequate for NSG

- Recommend:
  - Sanitize hands before gloving (washing)
  - Disinfect surfaces (laminar flow hoods, experimental equipment, floors, walls)
  - Sterilize tools (forceps, scissors, ear punches, etc), bedding, and cages
  - More frequently change cages
  - Monitor for pathogens frequently (swabbing)
Care and Handling of NSG mice: Personal Protective Equipment

- **Personal Protective Equipment (PPE)**
  - Sterile scrubs, gloves, dedicated shoes and shoe covers
  - Mask, goggles, hair/beard bonnet
  - Sterile smock
  - PAPR (Powered air purifying respirator): if necessary
Environmental Conditions: Suggestions

- Entry room/space (anteroom or dedicated hallway)
  - Clearly marked with tape and/or signage
  - Limited entry
  - Air shower (if available)
Environmental Conditions

- Sterilize or disinfect anything that may come into contact with the mice (autoclave or vaporized hydrogen peroxide (VHP))
- Use laminar flow hoods (or biosafety cabinet working with human pathogens) whenever possible
- Micro-isolator/ individually ventilated cages (IVCs)
  - HEPA (High-efficiency particulate absorption) filtered
- Cage Changes:
  - More frequent
  - Perform in disinfected laminar flow hood
  - Disinfect gloved hands and anything goes into the hood
  - Disinfect forceps (Wescodyne) between cages
  - If hands leave hood, disinfect again
NSG Mice at JAX: 
Barrier Conditions

Maximum barrier at JAX:

- Sterilized individually ventilated caging
- Sterilized feed and drinking water
- Air shower entry
- Change into clean room processed scrubs, smock and shoes
- Gloves, air hat or mask, cap and face shield
- Under maximum barrier conditions at JAX, bacterial disease in NSG are uncommon – less than 1% of mice >200 days

Overview of barrier levels at JAX: http://jaxmice.jax.org/health/barrier.html
Experimental Procedures and Transport

- Disinfect experimental equipment and environment (especially shared)
- Sterilize smaller tools (by autoclaving)
- MAP test and/or culture material prior to implantation to ensure sterility
- Use secondary containment during transportation
  - Plastic bag
  - Sterile smock
Breeding NSG mice

NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (005557)

- Homozygote/homozygote female x homozygote/hemizygote male
- No need to genotype pups, but genotype breeders
- Rotate every 8-9 months
- Replace if no litters in 60 days or if appear sick
NSG Mice
Care and Husbandry Suggestions

• Food
  ○ Autoclaved and/or irradiated

• Water
  ○ Acidified to pH 2.5 - 3.0 with HCl (or chlorinated) to control for *Pseudomonas* spp.
  ○ Autoclaved
  ○ *No antibiotics added routinely*

• Pathogens monitored
  ○ Directly (infected mice)
  ○ Indirectly (sentinel mice)
Pathogen monitoring

- Sentinel mice are those used for the detection of pathogens present in the room, and include:
  - Dirty-bedding (mostly fecal-oral)
  - Cage-contact (direct contact, aerosol, urine, fecal-oral)
  - Exhaust air (aerosolized)

- Use both immunocompetent and immunodeficient (including NSG periodically) as sentinels

For more information, please visit http://jaxmice.jax.org/jaxnotes/archive/497j.html
Health Monitoring Considerations

- Expanded health surveillance to detect all organisms excluded from barrier, including opportunistic bacteria
  - Shedding often intermittent
  - ↑ Non-lethal monitoring, e.g., fecal or oropharyngeal swab culture of colony mice desirable

- Immune competent mice may be transient carriers of opportunists that cause significant disease in immune deficient (e.g., Corynebacterium, Pneumocystis)
  - Testing may fail to detect due transient nature
  - Test immunodeficient mice directly

- Direct tests (e.g., culture, PCR), not serology, for severely immune deficient
Immunodeficient Mice at JAX: Animal Health Reports

Production Facility Animal Health Report

AREA: AX-27
Report # 1114AX27

AX-27 IS OPERATED AS A:
- Maximum Barrier
- High Barrier
- Standard Barrier

Please consult our website for descriptions of our Barrier levels.

ORGANISMS EXCLUDED FROM ALL BARRIERS (SHIPPING STOPPED)
If any of these organisms are found in any Production area, all shipments from the area are suspended and customers will be notified.

Organism | Sample Tested | Test Method | Nov 24 '14 | Oct 13 '14 | Sep 1 '14 | Jul 21 '14 | Previous 12 months
--- | --- | --- | --- | --- | --- | --- | ---
VIRUSES

| Organism | Sample Tested | Test Method | Nov 24 '14 | Oct 13 '14 | Sep 1 '14 | Jul 21 '14 | Previous 12 months
--- | --- | --- | --- | --- | --- | --- | ---

VIRUSES

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--- | --- | --- | --- | --- | --- | --- | ---

BACTERIA & MYCOPLASMA

| Organism | Sample Tested | Test Method | Nov 24 '14 | Oct 13 '14 | Sep 1 '14 | Jul 21 '14 | Previous 12 months
--- | --- | --- | --- | --- | --- | --- | ---

Health Status Reports: http://jaxmice.jax.org/health/index.html
NSG Mice

Treatment of infection

- Antibiotic treatment of *individual* mice:
  - Baytril (enrofloxacin)
    - 5-20 mg/kg SC as directed by your veterinarian
  - Others (amoxicillin, cephalexin)
  - No evidence of effectiveness as prophylactic treatment

- *Pneumocystis* containment
  - Sulfa-Trimethoprim: incorporate in feed or water
    - 50 mg/kg/day trimethoprim + 250 mg/kg/day sulfamethoxazole
  - Significant decrease in mortality
    - Fungi may or may not be detectable by histopath, but continue to be detectable by PCR

- Rederivation to eliminate opportunists from colony

Slate AR., et al., JAALAS. 2014.
Humanization

Health Considerations

- Possible human pathogens require ABSL2 housing & BSL2 laboratory

- Possible mouse pathogens from human donor, e.g., LCMV
  - Test tissues (MAP test, and/or culture bio-materials)

- Graft vs. host disease – severely immune deficient mice “attacked” by human tissue / cell transplants
  - Hunched posture, ruffled fur, reduced mobility, tachypnea, diarrhea, weight loss and/or hair loss / skin lesions
  - Time course (days to weeks) depends on strain of mouse, irradiation preconditioning, and type(s) and numbers of human cells injected
Warning Signs

- Non-specific clinical problems
- Unthriftiness, diarrhea, wasting, sickliness
- Weight loss
- Weakness, lethargy
- Acute and/or premature death
- Breeding problems, including:
  - Embryonic death
  - Small litters
  - Small, weak, and/or sickly pups
  - Pup mortality
Work With Your Veterinarian!

- When breeding performance suddenly declines
- Mice appear unhealthy (ruffled fur, hunched, reduced mobility/activity)
- Spontaneous death
- Positive culture/sentinel mice results
Summary

A little prevention goes a long way!

Handling and Care of the NSG Mouse
Thank you!

In need of mouse breeding and colony management expertise to advance your research?

Contact your regional representative today
www.jax.org/jaxmice/support/regionalcontacts

Contact technical support
www.jax.org/jaxmice/support/techsupport-index

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