Lab Animal Health Monitoring

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Routine Health Monitoring

- Why to do it?
- What to do?
- When to do it?
- Where/who to do it?
- How to interpret the test results?
- What should we do after infectious agents are detected? (inputs from lab animal veterinarian)
Why To Perform Health Monitoring

- Public health problem
  - Zoonotic and human pathogens carried by animals
- Animal health problem
  - Pathogens fatal for animals
  - Pathogenic, but not fatal for animals
  - Opportunistic pathogens
- Affected research data
- Current animal problem to be solved
- Indicators of microbiologic status of an animal/colony
What To Do

- Routine health monitoring
  - Gross/behavior (Clinical signs) observations
  - Parasitology monitoring
  - Pathology monitoring
  - Serology testing
  - Molecular biology testing
  - Microbiology testing
When to Perform Health Monitoring

- Routine Health Monitoring
  - How frequently?
  - What assay?
  - How many animals?
    - Random samples
    - sentinels
- Problem solving
Who/Where to do it

- Gross/behavior monitoring (Clinical signs)
  - By animal care taker/user/diagnostic lab staff (lab animal center/diagnostic lab)

- Parasitology/serology/microbiology/pathology monitoring
  - By experienced diagnostic lab staff
  - By lab animal pathologist

- Result interpretation
How to do it

- Routine Health Monitoring
  - How frequently?
  - What assay?
  - How many animals?
    - Random samples
    - sentinels

- Problem solving
  - Important decision!
  - Discuss w/ lab animal veterinarian
Lab Animal Health Monitoring

- Behavior/activity observation
- Routine health monitoring
  - Parasitology monitoring
  - Pathology monitoring
  - Serology testing
  - Molecular biology testing
  - Microbiology testing
- Genetic monitoring/phenotyping
Behavior/Activity monitoring

- Behaviors in natural habitats
- Behaviors in good lab environments
- Behaviors in stress lab environments
- Behaviors of mutant/modified strain

- Posture
- Movement
  - Nude mutants (ICR nude) jump more
- Barbering
  - C57BL, C3H, CDF
- Aggression
  - male vs female
  - C57BL/10 vs CBA/Ca

*Relied on animal care staff/users/veterinarian*
Behavior/Activity monitoring

- Direct signs
  - Death, diarrhea, conjunctivitis, hunched posture, rough hair coat, weight loss, decreased activities, respiratory noise, swollen lymph nodes….

- Indirect signs
  - Reduced diet or water consumption…

- Changes in established reaction patterns
  - Anesthetic period, changed immunological patterns/data

*Relied on animal care staff/users/veterinarian
Behavior/Activity monitoring

- Most infections are subclinical
- Infectious agents are easily transmitted by subclinical infected animals

Diagnosis of the subclinical infectious diseases mostly relies on laboratory diagnostic methods!
Genetic Monitoring

- Genetic contamination
- Incomplete inbreeding/residual heterozygosity
- Genetic drift/substrain variation
- Genetically engineered strains
- Strains with mixed genetic background
  - Usu. performed in research institutes or in animal breeder companies
Phenotyping

- Phenotyping of Genetically Engineered Modified (GEM) Mice and Rats
  - An investigator may have created a knockout mouse intending to produce a model for diabetes, yet the mouse demonstrates a cardiovascular lesion
  - IL-k/o mice with typhlitis/colitis lesion
    Infectious disease?
    Abnormality caused by ‘Genetic modification’?
Phenotyping Methods

- **Primary assessments**
  - Clinical characteristics (activity, gross anatomy, reproduction, life span)
  - Pathology (clinical & histopathologic)

- **Secondary assessments**
  - Embryonic evaluation
  - Physiology
  - Biochemistry
  - Behavior
Routine Health Monitoring

- Parasitology monitoring
- Pathology monitoring
- Serology testing
- Molecular biology testing
- Microbiology testing
Parasitology

- **Diagnosis**
  - Based on morphology (frequently)
  - Molecular diagnosis (less frequently)

- **Ectoparasite**
  - fur between ears, shoulders, or hip (dorsal area)—fur mites

- **Endoparasite**
  - Mucosal/content smear—protozoa
  - Perianal Tape Test—pin worms
  - Fecal test—pin worms & tapeworms

Photos obtained from RADIL website
Parasitology

- Endoparasite
  - Tissue gross/histo exam
  - Cysticercoid cyst
    - Cysticercus fasciolaris (in liver, cyst of cat tape worm)
  - Trichosomoides crassicauda

Photos obtained from RADIL website
Routine Health Monitoring

- Parasitology monitoring
- Pathology monitoring
  - Infectious diseases
  - Non-infectious disorders
- Serology testing
- Molecular biology testing
- Microbiology testing
Pathology Testing

- **Histopathology**
  - Interpretation of tissue morphology (gross and histological lesions)
    - Disease diagnosis
    - Health monitoring

- **Clinical Pathology**
  - Clinical chemistry (liver, kidney, and multi-organ functions)
  - Hematology
  - Cytology

- Phenotyping of genetically-modified animals
Histopathology Testing

- Disease Diagnosis
  - Rely on typical histo lesions & staining characteristics
- Routine Health monitoring
Rodent Microbes

- Parvovirus
- Coronavirus
- Cytomegalovirus
- Sendai virus
- PVM
- LCM
- Mousepox
- Reovirus
- Hantavirus
- Herpesvirus
- Rotavirus
- Clostridium piliforme
- Mycoplasma pulmonis
- Pseudomonas aeruginosa
- Corynebacterium kutscheri & bovis
- Streptobacillus moniliformis
- Pasteurella pneumotropica
- Citrobacter rodentium
- Staphylococcus aureus
- Helicobacter spp.
- CAR bacillus
- Pneumocystis
- Proteus mirabilis
- Streptococcus
- Salmonella
- Chlamydia
- Klebsiella
- Dermatophytes
Gross Lesion

- SDAV/RCV infection (coronavirus)
  - Cervical swelling
  - Nasal/ocular discharges (porphyrin stained)

Photos obtained from RADIL & Merck websites
Histopathology

- SDAV/RCV infection
  - Inflammation of salivary glands and Harderian glands

Photos obtained from AFIP website
Histopathology

- Mouse Hepatitis Virus (MHV) (coronavirus)

Photos obtained from RADIL website
Histopathology

- Tyzzer’s Disease (*C. piliforme*)

Photos obtained from RADIL website
Histopathology

- *Helicobacter* spp.

Photos obtained from RADIL website
Histopathology

- Chronic Respiratory Disease
  - *Mycoplasma pulmonis*
  - Cilia-Associated Respiratory (CAR) Bacillus

Photos obtained from RADIL website
Histopathology

- Encephalitozoon cuniculi

Photos obtained from RADIL website
Histopathology

- Ectromelia virus (mousepox)

Photos obtained from RADIL website
Emerging Disease

- Rat Respiratory Virus (RRV)
  - A hantavirus
  - Idiopathic interstitial pneumonia of rat

Photos obtained from RADIL website
Clinical Pathology

- Urinalysis
  - Kidney, liver,
- Hematology & Clinical Chemistry
  - Species/strain variation
Typical gross/histopathology lesions are uncommon for most infectious agents.

Animal sacrifice is required.

- Serology/molecular biology/microbiology diagnostics are the choice.
- Histopath is the great method to identify new pathogens or disorders.
Routine Health Monitoring

- Parasitology monitoring
- Pathology monitoring
- **Serology testing**
- Molecular biology testing
- Microbiology testing
Serologic Test

- Traditional Assays
  - Enzyme-Linked ImmunoSorbent Assay (ELISA)
  - Indirect Fluorescent Antibody Test (IFA)
  - Hemagglutination Inhibition Assay (HAI)
Serology Testing

- Traditional Assays
  - ELISA
    - Ag-coated microplates (polystyrene or polyvinyl)
    - Primary Aby
    - Enzyme-conjugated secondary Aby
    - Chromogenic enzyme substrate
  * Sensitive, Specific?
Serology Testing (continual)

- Traditional Assays
  - IFA
    - Infected and uninfected cells fixed to wells in a glass slide
    - Primary Aby
    - Fluorescent labeled secondary Aby
  * Sensitive, Specific
Serology Testing (continual)

- **Traditional Assays**
  - **HAI**
    - The ability of specific Aby to inhibit virus-mediated hemagglutination
    - V-bottom microplate
    - Rodent parvoviruses, Reovirus type 3, Pneumonia virus of mice, Sendai virus
  * Low sensitivity, High Specificity
Serology Testing (continual)

- **Traditional Assays**
  - Advantage
    - Cheap equipment
    - Automated (ELISA)
    - Sensitive (ELISA, IFA)
    - Specific (IFA, HAI)
  - Disadvantage
    - Individual Assay (inefficient)
    - Manual (IFA & HAI) (labor-intensive)
    - Insensitive (HAI)
Improvement in Detection

- **Multiplex & Automation**
  - Multiplex Fluorescent Immunoassay (MFI)
    - Microspheres internally dyed with red and infrared fluorophores
  - 100 different microspheres

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<th>Aby α A</th>
<th>Aby α B</th>
<th>Aby α C</th>
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<tr>
<td>Result</td>
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</table>
Improvement in Detection (Continual)

- Automation & Multiplex
  - ElectroChemiluminescence
- Coated nitrocellulose membrane
- the light emitting chemiluminescent reaction is preceded by an electrochemical reaction.

Ru(bpy)$_2$ = BV-TAG$^{TM}$ Label
"Ru(bpy)$_2"$ = BV-TAG Label
TPA = Tripropylamine
Serology Testing (continual)

- MFI & ECL Assays
  - Advantage
    - Automated
    - Multiplex
    - Sensitive & Specific
    - Less amount of test article required
  - Disadvantage
    - Expensive equipment
Routine Health Monitoring

- Parasitology monitoring
- Pathology monitoring
- Serology testing
- Molecular biology testing
- Microbiology testing
Molecular Biology Diagnostics

- Application of molecular biology methodologies in nucleic acid detection
- Monitoring
  - The nucleic acids from microbes (virus, bacterium, parasite)
  - The genetic materials from biotics
  - The expression status of genes (mRNA)
Molecular Biology Methodologies

- **Nucleic acid amplification**
  - PCR, RT-PCR, nested PCR (in test tubes)
  - *In-situ* PCR, *in-situ* RT-PCR

- **Nucleic acid hybridization**
  - Southern blotting (detecting DNA)
  - Northern blotting (detecting RNA)
  - *In-situ* hybridization

- **Sequencing**

- **Restriction enzyme digestion**
  - Restriction fragment length polymorphism (RFLP)
Nucleic acid amplification

- PCR
- RT-PCR
- In situ PCR
- In situ RT-PCR

Forward Primer

Reverse Primer

1\textsuperscript{st} cycle

\(2^{n-1}\) amplicon

\(n=30\)

\(10^9\) amplicon
Nucleic Acid Hybridization

- Southern blot
- Northern blot
- *In situ* hybridization
  - DNA
  - RNA

Target gene

Add probe
Molecular Biology Methodologies

- Sequencing
  - Time consuming
  - Cost
  - Effort

- Restriction digestion (RFLP)

Strain A: E E E
Strain B: E E

E: EcoRI

A B
Molecular Biology Diagnostics

- Goals
  - Sensitive
  - Accurate
  - Fast

★ Amplification
★ PCR/RT-PCR
PCR/RT-PCR

- Primer design
  - Generic v.s. Specific
  - Conserved region v.s. Diverse region

EX. Rodent parvoviruses:
  - Generic rodent parvovirus PCR
    NS region: ~90% similarity
  - MMV, MPV, KRV, H-1 specific PCR
    VP region: ~70-75% similarity
PCR/RT-PCR

- Specimen quality
  - Clinical samples
    - Feces, urine, blood, bio-swab, tissues
      - Inhibition factors
  - Cultural & environmental specimens
    - Cells, media, cage swab
      - Amount of specimen to screen
      - Inhibition factors
  
  ❖ False Negative!
Rodent Parvoviruses

- **Mouse**
  - Mice Minute Virus (MMV)
  - Mouse Parvovirus 1 (MPV-1)

- **Rat**
  - Kilham Rat Virus (KRV)
  - Toolan’s H-1 Virus (H-1)
  - Rat Parvovirus 1 (RPV-1)
  - Rat Minute Virus 1 (RMV-1)

- **Hamster**
  - Hamster Parvovirus (HaPV)
Diagnosis of Parvovirus Infection

- PCR assays (Mesenteric LN, Spleen)
  - Generic PCR assay
  - Serotype specific PCR assays
    - KRV specific
    - H-1 specific
    - RMV-1 specific
    - MMV specific
    - MPV/HaPV specific

RMV-1 Specific
Rodent Coronaviruses

- **Mouse**
  - Mouse Hepatitis Virus
    - Multiple strains
      - Polytrophic (respiratory): JHM, 1, 2, 3, A59, S
      - Usually more virulent
    - Enteric: D, Y, DVIM
    - Highly mutable (esp. the S gene)
    - Pathogenic vs. nonpathogenic

- **Rat**
  - Rat Coronavirus/Sialodacryoadenitis virus (RCV/SDAV)
    - Multiple strains
Rodent Coronavirus

- Linear, ss(+) RNA
- **Generic** RT-PCR
  - Both MHV & RCV/SDAV
  - MHV: feces or mesenteric LN
  - RCV/SDAV: Salivary gland or Harderian gland or lung
- ✷ No virus species/strain-specific RT-PCR
Helicobacter in Rodents

- Gram-negative, spiral
- Enterohepatic infection
  - \( H. \text{ hepaticus} \)
  - \( H. \text{ bilis} \)
  - \( H. \text{ typhlonius} \)
  - \( H. \text{ rodentium} \)
- Hepatitis, enterocolitis, hepatocellular neoplasmas
- Model for \( H. \text{ pylori} \)
Enterocolitis

Photos obtained from RADIL website
# Helicobacter Generic PCR

- **Consensus region of helicobacters**
- **ß-Actin PCR**
  - Housekeeping gene
  - Extraction control
  - Inhibition factors monitor
- *Fecal PCR*

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<thead>
<tr>
<th>hepa</th>
<th>bilis</th>
<th>typh</th>
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<tr>
<td>$10^2$</td>
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<td>$10^2$</td>
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<tr>
<td>10</td>
<td>10</td>
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</table>

**Helicobacter:** 375 bp  
**ß-Actin:** 272 bp
Helicobacter Species-specific PCR

- *H. hepaticus* specific
- *H. bilis* specific
- *H. typhlonius* specific
- *H. rodentium* specific

* Fecal PCR

![Image of gel electrophoresis](image.png)

- *H. hepaticus*: 396 bp
- Sensitivity: 10 copies
Improvement in Amplification

- **Multiplex PCR**
  - ↑ information for client dollar
  - ↑ efficiency

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<thead>
<tr>
<th>Template copies</th>
<th>10³</th>
<th>10²</th>
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<th>Fecal Control</th>
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<tr>
<td>H. hepaticus</td>
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<tr>
<td>H. typhlonius</td>
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<tr>
<td>H. bilis</td>
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Signal Amplification

- PCR-ELISA

**PCR**
- Forward Primer
- Reverse Primer
- n\textsuperscript{th} cycle

**ELISA**
- Anti
- Antibody

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Add substrate.
The amount of product is directly related to the amount of specific antibody present.

Add enzyme-labeled antigen.
This binds to the antibody.

Wash
Signal Amplification

- PCR-Oligo hybridization

PCR

Hybridization

Forward Primer

Reverse Primer

n^{th} cycle
Signal Amplification

- PCR- RFLP

**PCR**
- Forward Primer
- Reverse Primer
- n\textsuperscript{th} cycle

**RFLP**
- Strain A
  - EcoRI
- Strain B
  - EcoRI

E: EcoRI
Improvement in Detection

- Automation
  - Labeled Probe
    - TaqMan
      - Quantitative Real-time v.s. endpoint PCR
      - The fluorogenic 5' nuclease PCR assay
        - the 5' --> 3' nuclease activity of Taq
        - the emission of the reporter dye is quenched by the intact quencher
Improvement in Detection

- Automation & Multiplex
  - Multiplex Fluorescent Assay
    - Microspheres internally dyed with red and infrared fluorophores
  - 100 different microspheres

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<th>A</th>
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Improvement in Detection (Continual)

- Automation & Multiplex
  - ElectroChemiLuminescence
  - Oligo-coated membrane
  - the light emitting chemiluminescent reaction is preceded by an electrochemical reaction.
Emerging Disease

- Murine Norovirus
  - Related to human Norwalk virus
  - Primary GI infection
  - No clinical signs in naturally infected mice
Emerging Disease

- Murine Norovirus exp. inoculation
  - RAG/STAT-/- mice exhibited high Mt with encephalitis, meningitis, vasculitis, pneumonia, hepatitis
  - RAG-/- exhibited low Mt but persistently infected
  - Immunocompetent mice seroconverted and transiently infected
  - Mice with no INF$_{\alpha\beta}$ and IFN$_{\gamma}$ receptors are more susceptible to MNV infection.
Microbiology monitoring

- Isolation of microbes in pure culture
  - Routine monitoring area: Nasopharynx and cecum (rodents and rabbits)
  - Area of suspected lesions
- Some organisms require specific culture medium and conditions
Culture Methods

- Sensitive and expressive
- Results are dependent on culture media, experience,…
- Expensive, sometimes time consuming and labor-extensive
- For most bacteria and fungi: the best method
Techniques for Definite Diagnosis of Rodent Infections

- **Positive results: What can be done**
  - Confirm lab results
    - Retest the same sample
    - Retest with other method
    - Retest in other diagnostic lab
    - Retest samples from the same source
  - Rule out the cross-reaction
    - Cross reactivity with related organisms
How to Interpret the Results

- Possible combinations of results of different testing (e.g. serology and PCR for MPV)

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<tr>
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<th>MPV PCR</th>
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</tr>
<tr>
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<td>-</td>
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Routine Health Monitoring

How frequently to screen?
What assay/method to be applied?
How to interpret the results?