**Pre-Analytical Variables That Affect Interpretation**
- Collection site and method
- Sex
- Age
- Strain and origin
- Fasting vs feeding
- Time of day circadian rhythms

**Blood Collection**
- Manual Restraint
- Chair restraint
- Squeeze cage
- Voluntary presentation
- Chemical Restraint
  - Ketamine, telazol, medetomidine
  - Inhalation

**Manual restraint**
- Epinephrine and glucocorticoid release
  - May interfere with:
    - White blood cell populations
    - Cortisol levels, alkaline phosphatase
  - Splenic contraction
  - Hyperglycemia
  - New world monkeys, rodents

**Epinephrine vs Glucocorticoids**
<table>
<thead>
<tr>
<th>Excitement</th>
<th>Stress Leukogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils ↑</td>
<td>Neutrophils ↑</td>
</tr>
<tr>
<td>Lymphocytes ↑</td>
<td>Lymphocytes ↓</td>
</tr>
<tr>
<td>Monocytes ↑</td>
<td>Monocytes ↑</td>
</tr>
<tr>
<td>Eosinophils ↓</td>
<td>Eosinophils ↓</td>
</tr>
</tbody>
</table>

**Effects of Ketamine**
- Muscle necrosis
- Fibrosis
- Histiocytic inflammation
- Clin Path
  - CK
  - LDH
  - AST
  - ALT

Sun, F. Cont Top Lab An Sci, 42, 2003
Voluntary Presentation

Diabetic Monitoring
Lancet bleeding vs Venous bleeding

Blood Collection
- Standardize the collection site and method
  - Cardiac
    - Elevated P and K
    - Increased muscle enzymes
  - Anesthetics
    - Isoflurane minimally stressful
    - Generally CO₂ asphyxiation and pentobarbital stressful

Blood collection
- Anesthesia method
  - CO₂ administration in rodents

Venous Blood Gas measurements
- Cannot accurately measure TCO₂ (bicarbonate)
- Increased potassium – due to metabolic acidosis

Arterial Blood Gas measurements
- Increased lactate
- Increased pCO₂
- Decreased pH

Non-terminal Bleeding in Mice
- Submandibular vein
- Facial vein
- Retro-orbital venous plexus/sinus – this technique has been largely replaced by the submandibular vein
- Saphenous venipuncture
- Modified tail-clip

Submandibular Vein

Jerry Silverman
“Using Nomina Anatomica Veterinaria as a guide, there is no submandibular vein in the mouse.”
JAALAS, 49:4, p.400, 2010

Non-terminal Bleed in Rats
- Jugular vein
- Sublingual vein
- Tail vein

Braintree Scientific
Blood Collection for Facial Vein (Submandibular) Blood Samples
Medipoint, Inc.
Facial Vein and Submandibular Vein meet at the rear end of the Mandibular Bone, convenient and consistent source of blood

Collection site

Evaluation of Saphenous Venipuncture and Modified Tail-clip Blood Collection in Mice

Abatan, O.I. et al, JAALAS, 47:3, 8-15, 2008

Blood Collection

- Hemolysis
  - Mild hemolysis (1+)
    - generally non-significant
    - very common in rodent samples
  - Moderate to Severe (2+ to 5+)
    - Potassium, phosphorus and bilirubin elevated along with other analytes

- Remember: Always remove needle before injecting blood into collection tube to avoid hemolysis

EDTA Contamination

- When transferring blood to EDTA first, there is a chance for contamination and transfer to the clinical chemistry tube
- EDTA binds to calcium, and prevents it from being measured

Selected Data From a Rat Necropsy Control Females

<table>
<thead>
<tr>
<th>Animal #</th>
<th>BUN</th>
<th>Calcium</th>
<th>Creatinine</th>
<th>Glucose</th>
<th>Potassium</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>11.9</td>
<td>0.3</td>
<td>170</td>
<td>6.3</td>
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<tr>
<td>2</td>
<td>16</td>
<td>11.3</td>
<td>0.3</td>
<td>167</td>
<td>7.2</td>
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<tr>
<td>3</td>
<td>19</td>
<td>6.3</td>
<td>0.3</td>
<td>135</td>
<td>8.1</td>
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<tr>
<td>4</td>
<td>21</td>
<td>4.2</td>
<td>0.3</td>
<td>156</td>
<td>10.2</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>10.8</td>
<td>0.3</td>
<td>221</td>
<td>6.1</td>
</tr>
</tbody>
</table>

What is the cause of these changes?
EDTA
- Advantages
  - Ideal for cellular morphology
  - Inhibits bacterial proliferation
- Disadvantages
  - Cannot be used for some chemistry analytes
  - Underfilled tubes – RBCs shrink due to hypertonicity

Lithium Heparin
- Advantages
  - Can also be used for clinical chemistry
  - For small animals with limited blood, collection into a single tube minimizes loss.
  - Do not have to wait for sample to clot
- Disadvantages
  - Morphology not ideal
  - Interferes with some immunoassays

Sodium Citrate
- 3.2% vs 3.8%
- Both can be used, however
  - The milder chelation of 3.2% provides greater accuracy of coagulation results
  - Cannot compare data if collected into different %

Blood Collection
- Processing Times
  - Serum must sit for 30 minutes, minimum to allow to fully clot
  - If plastic tubes are used, many need to sit for at least 1 hour if they do not contain a clotting additive
  - Delayed processing alters analytes
    - e.g. Decreased glucose
  - Freezing and Thawing

Sex
- Example: Rodents
  - Males
    - RBC count, hemoglobin and hematocrit are generally lower

Sex
- Examples: Nonhuman primates
  - a) Old world monkeys – menses in females
    - Decreased indices of RBC mass
    - Can become chronic
  - b) Muscle mass significantly less than males
    - Increased CK, BUN, creatinine
  - c) Great apes and baboons – sex swelling
**Sex Swelling**

- **Follicular phase**
  - Swelling contains 1-2L of fluid
  - Plasma volume contracts
- **Luteal phase**
  - ↓ RBC count
  - ↓ Hct and hemoglobin
  - ↓ WBC count

**Rhesus Monkey**

- Sex Skin Swelling

**Age**

- Young animals
  - Elevated phosphorus
  - Elevated ALP
  - Elevated GGT (non-human primates)

**Strain**

- Cynomolgus monkey
  - Mauritian vs Southeast Asian origin
  - Rhesus monkey
  - Indian vs Chinese
  - Owl monkey
  - *Aotus nancymae* vs *Aotus azarae*

**Mauritian Cynomolgus Monkeys Hematology**

- Increased RBC count
- Decreased reticulocytes
- Decreased MCV
- No change in PCV and hemoglobin
- Decreased WBC count

Zuehlke, et al. in Novel Approaches Towards Primate Toxicology, 2006
Mauritian Cynomolgus Monkeys serum biochemistry

Zuehlke, et al. in Novel Approaches Towards Primate Toxicology, 2006

Indian vs Chinese

Differences
- Genetic
- Morphometric
- Behavior
- Response to disease

Origin
- Wistar Rats – 2 different suppliers
  - Significant differences
    - WBC, RBC, platelet, lymphocyte, monocyte, eosinophil, reticulocytes, MCV, MCH, MPV, plateletcrit
    - Example: Lymphocytes
      - Breeder 1 = 3.0 ± 0.1
      - Breeder 2 = 8.1 ± 0.1


Abnormal Cytogram

Advia Hematology Analyzer
Sprague-Dawley Rat
Fisher Rat
Wistar Rat

Fasting
- Minimal changes in hematology
- Select changes in serum chemistry
  - Decreases in:
    - Glucose
    - Triglyceride
    - Cholesterol
    - ALKP and ALT
  - Increases in:
    - Creatinine

Advia 120 and 2120 – Wistar Rats
- Advia – most common hematology analyzer used for rodents
- 5-20% of a Wistar group may be affected
- Parameters affected
  - Increased RDW
  - Increased MCV
- Recent study by Charles River
  - Mild in vitro agglutination of RBCs
  - Occurs with RBC diluent but not reticulocyte diluent

Hematology Case #1
- 7-month old male rhesus
- Pale haircoat
- Alopecia
- Seemed to be less active
- PCV = 5%
  - Non-regenerative
  - Normocytic, normochromic
  - Morphology - unremarkable

White Monkey Disease
- Cage treated for rust several months prior
- Zinc toxicosis
- Decreased copper
- Severe, non-regenerative anemia
- Hairloss and depigmentation
- Baboons, macaques – young animals only

Rodent Erythrocytes
- As compared to Dogs
  - More polychromasia (1-6% circulating RBCs or 100,000-350,00/µl)
  - More anisocytosis
  - Occasional Howell Jolly bodies
  - Occasional nucleated red blood cells

Preparation of Blood Smears from Rats
- Low intra-erythrocytic ATP levels
- Highly susceptible to crenation and poikilocytosis in vitro
- Blood smear preparation ASAP after collection. Ideally within 30 mins

Interpreting Decreased RBC Production in Rodents
- Most species
  - Indices not changed as few reticulocytes normally in circulation
- Mice/rats
  - Decreased MCV
  - Decreased RDW
  - Increased MCHC
  - Short RBC life span
    - Anemia of chronic disease develops more quickly

Normal Erythrocyte Life Spans
<table>
<thead>
<tr>
<th>Species</th>
<th>Mean diameter (µm)</th>
<th>Mean life span(days)</th>
<th>Retics – (× 10^3/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>5-7</td>
<td>41-52</td>
<td>150-388</td>
</tr>
<tr>
<td>Rats</td>
<td>6-7</td>
<td>56-69</td>
<td>114-399</td>
</tr>
<tr>
<td>Rabbits</td>
<td>7</td>
<td>57</td>
<td>100-250</td>
</tr>
<tr>
<td>Pigs</td>
<td>6</td>
<td>86</td>
<td>&lt; 80</td>
</tr>
<tr>
<td>Rhesus</td>
<td>7</td>
<td>92-100</td>
<td>&lt; 80</td>
</tr>
</tbody>
</table>
**Rodent – erythrocyte parasites**
- *Plasmodium berghei*
- Many experimental mouse models
- Young Wistar rats highly susceptible
- *Mycoplasma coccoides (Eperythrozoon coccoides)*
  - Mice and rats
  - May cause anemia

**The Belgrade Rat**
- A hypochromic, microcytic anemia
- Abnormal iron metabolism
- Missense mutation in divalent metal transporter 1
- Decreased iron absorption particularly from the duodenum

**Rodent Leukocytes**
- Lymphocytes
  - Predominant cell
- Neutrophils
  - Often look hypersegmented
  - Low number of ring forms normal, increase with neutrophilia
- Eosinophils
  - Low numbers in peripheral blood
  - Can also form bands
- Basophils
  - Rarely seen in peripheral blood

**Leukocyte Counts in Rodents**
- Less robust in as compared to large animals
- Magnitude of changes (dog > rat > mouse)
- Qualitatively similar effects with excitement and stress
- Even small changes may be significant
- Inflammation in rodents
  - Increased neuts and lymphs, +/- monocytes
  - Left shifts in rodents less common than in larger animals
  - But morphology of the shift when present is similar
- Decreased neutrophils
  - Difficult to evaluate when resting count is so low

**Rodent Platelets**
- “Sticky” and clump easily

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Dog</th>
<th>Mouse</th>
<th>Macaque</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>893-1340</td>
<td>241-561</td>
<td>453-1181</td>
<td>318-672</td>
</tr>
</tbody>
</table>
Rodent Hematopoiesis
- Bone marrow
  - Lymphocytes ~1/5th to 1/3rd of cells
  - Megakaryocytes more numerous
  - May play an active role in removing neutrophils from circulation
- Spleen
  - Reservoir for hematopoiesis
  - Output is about 50% of the bone marrow under normal conditions
  - Megakaryocytes normally in clusters in the red pulp

Mouse – Bone Marrow
- Neutrophilic ring forms

African Green Monkey
What is this cell type?

Large Granular Lymphocytes
- Normally – 2-3% of circulating cells or less but you will see them in normal blood smears
- NK cell or CD8+ lymphocyte (cytotoxic T cells)
- Preformed granules
  - Perforin
  - Granzymes (proteases)
- Rapid response to viruses and other organisms (e.g. Rickettsia) and tumor formation

Large Granular Cell Leukemia Aged Fisher 344 Rats
- Frequently associated with an immune-mediated hemolytic anemia
- Neutrophilia with a left shift
- Moderate to severe thrombocytopenia
- Altered hemostasis
- Splenomegaly
- Chemistry changes consistent with hepatic infiltration

Hamster
- Very similar to mouse and rat
- Hibernation
  - Normal RBC lifespan ~ 50-70 days --Increases to 160 days
  - RBC counts and hemoglobin levels higher
  - WBC counts decrease
Hematology Case #2: 2-month-old Syrian Hamster

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Results</th>
<th>Ref Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC x10^6/μL</td>
<td>3.7</td>
<td>4.0-10.0</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>5.0</td>
<td>13.0-19.0</td>
<td></td>
</tr>
<tr>
<td>Hematocrit %</td>
<td>16</td>
<td>39-59</td>
<td></td>
</tr>
<tr>
<td>MCV fL</td>
<td>91</td>
<td>88-75</td>
<td></td>
</tr>
<tr>
<td>MCHC g/dL</td>
<td>37</td>
<td>28-37</td>
<td></td>
</tr>
<tr>
<td>WBC x10^3/μL</td>
<td>12.0</td>
<td>6.5-11.0</td>
<td></td>
</tr>
<tr>
<td>Seg neutrophils x10^3/μL</td>
<td>3.0</td>
<td>2.9-3.1</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes x10^3/μL</td>
<td>5.4</td>
<td>4.4-7.2</td>
<td></td>
</tr>
<tr>
<td>Platelets x10^3/μL</td>
<td>573</td>
<td>500-400</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td>Mod hemolysis</td>
<td></td>
</tr>
</tbody>
</table>

Babesiosis

Hamsters have been used experimentally to study Babesia spp. and as a tool for diagnosing human babesiosis (Babesia microti) through inoculation.

Rabbits
- Heterophils (also termed neutrophils or pseudoeosinophils)
- 2-4% polychromasia
- Lymphocytes and neutrophils approximately equal in #
- Leukocytosis often absent in bacterial injections

University of Georgia, College of Vet Med

Guinea Pigs
- Heterophils predominante
- Kurloff cells
  - More common in females
  - Presumed to be an NK cell

Pig Hematology
- Rouleaux
- Crenation and poikilocytosis are common findings
- High sedimentation rates
- Highly susceptible to hemolysis by hypotonic saline
- Little central pallor
- Lymphocytes more common than neutrophils
  - e.g. Yucatan minipig, 53% lymph, 37% neuts
  - Band cells in normal pigs

Iron Deficiency Young Pigs
- Rapid growth rate
- Sow’s milk contains insufficient iron
- Not raised on soil which is a normal source of iron
Sheep
- Small erythrocytes
- Small platelets
  - Some hematology analyzers cannot distinguish platelets from erythrocytes

Sheep
- *Mycoplasma ovis*
  - Erythrocytic
  - Cause of hemolytic anemia in sheep and goats

Deer
- Sickled erythrocytes
  - Non-pathogenic
  - Common

Macaque
*Plasmodium inui*
- Ring trophozoites
- Hemazoin – malaria pigment

Model of Human Malaria
- Great Apes
  - Require splenectomy to maintain *Plasmodium falciparum*

- Current models
  - Squirrel monkey
  - Owl monkey

Bone Marrow – Rhesus Macaque
Hematology Case #3
24 February 2012
- 9 month old male squirrel monkey
- Presented for wound on foot
  - Presumed to be a result of fighting
- Draining inguinal lymph node enlarged
- CBC to check WBC count
  - Normal

BABESIA MICROTI

HEMOPLASMAS

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Anaplasma platys</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Anaplasma phagocytophilum</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Bartonella henselae</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Bartonella vinsonii</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Babesia canis</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Babesia species (non-canis)</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Ehrlichia canis</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Ehrlichia species</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Mycoplasma hominis</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Neorickettsia risticii</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Rickettsia rickettsii</td>
<td>Negative</td>
</tr>
<tr>
<td>Ab Titer Lyme disease</td>
<td>1:256 (Pos)</td>
</tr>
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</table>

ZOOLOGIX – PCR TICK PANEL

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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<td>PCR Neorickettsia risticii</td>
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</tr>
<tr>
<td>PCR Rickettsia rickettsii</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Babesia species (non-canis)</td>
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</tr>
<tr>
<td>PCR Babesia canis</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR Bartonella henselae</td>
<td>Negative</td>
</tr>
<tr>
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<tr>
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</tr>
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<td>Negative</td>
</tr>
</tbody>
</table>

Ixodes Ticks
- Deer Ticks
- Vector for:
  - Lyme Disease
  - Babesia microti
  - Studies on ticks show 10% incidence of co-infectious
**White Blood Cell**

**Platelet**

**Extracellular Structure**

**Protruding from within the Erythrocyte?**

---

**TRANSMISSION ELECTRON MICROSCOPY**

**Intracellular Structure**

**Intracellular Structure**

---

**Additional Diagnostics**

- Repeat serology at Antech - **POSITIVE**
- Repeat serology at a different laboratory (Tulane primate center) - **NEGATIVE**
- Repeat PCR for Babesia on a second sample taken 3 weeks after the first - **NEGATIVE**
- Determine possible avenues of tick exposure
  - Large tick population
  - History of a technician with Lyme's disease
- Nucleic acid from RBC pellet sent to a parasitology lab for Babesia/Theileria PCR - **NEGATIVE**
What Next?
- Do we have parasites?
- Do we have a protein that is cross-reacting with one of the assays for Borrelia?

Additional primers for other parasites – positive for Mycoplasma!!

The 16S rRNA gene sequence shows 99% identity with Candidatus Mycoplasma kahanei nov. sp.

Rhesus Monkey
University of Texas Health Science Center
- 20 year-old, male
- Housing - last 6 years indoor, prior was part outdoor
- 5 years ago
  - Hct 16%, High MCV and MCHC, increased bilirubin
  - RBC morphology showed “abnormal vacuoles”
  - Has had a few recurrent episodes of anemia
  - Blood collected early in 2014, routine

Current plans
- Blood sent to Texas A&M for PCR identification
- Most likely diagnosis
  - Candidatus Mycoplasma haemomacaque

Coagulation in Rodents
- Problems and Pitfalls
  - Rodents are resistant to thrombus formation
  - Standard coagulation biomarkers not very sensitive or specific
  - Endpoints not standard in early screening studies
  - Abnormalities in longer term studies often subtle
  - Histopathology not sensitive
  - Results easily altered by collection techniques

Coagulation Panel - Rodents
- Routine
  - Absolute Platelet count
  - Prothrombin time (PT)
  - Activated partial thromboplastin time (APTT)
- Variable
  - Fibrinogen
  - MPV
  - Bleeding time
    - Distal tail incision, normal less than 60 seconds
  - D-dimer, ATIII can be helpful but not well characterized
  - Buccal mucosal bleeding time – foot stick or tail clip
Spontaneous Coagulopathy in Inbred WAG/RijYcb Rats

- Elevated APTT
- Normal PT
- Normal platelet


Gene deletion results in an intrinsic pathway defect

Knock-OUT Mouse: FVB Background

- No spontaneous hemorrhage
- Routine tail clip for genotyping
- Uncontrolled bleeding leading to death

- Elevated APTT
- Normal PT
- Normal platelet

Coagulation – Pregnant Rat

- Suitable model for humans in late pregnancy
  - Increased fibrinogen
  - Increased platelet counts
  - Decreased PT
  - Overall activity of vitamin-K-dependent coagulation factors
  - Increased APTT
  - Increased ATIII

CLINICAL CHEMISTRY OF LABORATORY ANIMALS

Kirstin Barnhart, DVM, PhD, DACVP
M. D. Anderson Cancer Center

Glucose

- Mice
  - Higher in cardiac puncture as compared to jugular
  - Anesthetic method has been reported to affected results
  - Normally higher than large mammals
  - Average between 125 and 250 mg/dl after 4hr fast
  - Diabetes – defined as greater than 300 mg/dl
  - Must be persistent!
  - Common to have elevations greater than this with stress

- Rats
  - Lower average fasted levels, around 100 mg/dl

GLUCOSE

Old World monkeys
- 20-30 mg/dl lower than humans
- 100-126 mg/dl likely to be overt diabetes

New World monkeys
- Many species are similar to rodents and have marked elevations due to stress
- Glucose greater than 200 mg/dl is common in squirrel and owl monkeys
Type II Diabetes Animal Models

- C57Bl/6J ob/ob
  - Obese, hyperglycemic, hyperinsulinemic
- Zucker fatty rat (fa/fa)
  - Obese, hyperinsulinemic, normoglycemic
- Ossabaw pig
  - Metabolic syndrome
- Rhesus and cynomolgus monkeys
  - Insulin resistant
  - Overt type II diabetes

MORBID OBESITY

Methods for Assessing Function of Pancreatic Islet Cells

- Oral Glucose Tolerance Test (OGTT)
- Intravenous Glucose Tolerance Test (IVGTT)
- Euglycemic Clamp

OGTT
- Better than a fasting blood glucose
- Best used as a screening test
- Plagued by many factors that cause poor reproducibility
- 20% non-diagnostic
- Must be confirmed with a repeat test

IVGTT
- Indirect measure of insulin sensitivity
- Using differential equations (Minimal model) can measure insulin sensitivity/resistance

PICC Line = Peripherally Inserted Central Venous Catheter
Enzymes - Hepatocellular injury

<table>
<thead>
<tr>
<th>Enzyme and Abbreviation</th>
<th>Liver Specific?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine Aminotransferase (ALT)</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Aspartate Aminotransferase (AST)</td>
<td>No</td>
</tr>
<tr>
<td>Sorbitol Dehydrogenase (SDH)</td>
<td>Yes</td>
</tr>
<tr>
<td>Glutamate Dehydrogenase (GLDH)</td>
<td>Yes</td>
</tr>
<tr>
<td>Ornithine Carbamoyltransferase (OCT)</td>
<td>Yes</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH)</td>
<td>No</td>
</tr>
</tbody>
</table>

Alanine Aminotransferase
- Largely liver specific
- Muscle injury can cause mild elevation
- Hemolysis
- Anticonvulsants and steroids
- In the liver, increase occurs from
  - Traumatic injury
  - Inflammation
  - Neoplasia
  - Hypoxia
  - e.g. severe anemia

Aspartate Aminotransferase
- High activity in:
  - Liver
    - Response to injury generally less than ALT
    - Declines faster
  - Muscle (cardiac and skeletal)
  - Erythrocytes
    - Increased with hemolysis

Alkaline Phosphatase
- Located on the cell membrane and microsomes - Induced
- Cholestatic disorders in all species with variable sensitivity
  - Dog > rat > monkey
- Elevated in young animals
- Bone isoenzyme
- Species differences
  - Rats have abundant intestinal isoenzyme
  - Increases after meal
  - Decreases with fasting
  - Steroid inducible isoenzyme in dogs
- Large animals
  - Insensitive
  - Cholestatic diseases very uncommon

Gamma Glutamyltransferase
- Sources
  - Biliary epithelium
  - Proximal renal tubules
    - Increased in urine with renal injury but not serum
  - Mammary gland
    - Increased in colostrum of all species EXCEPT horses
- Located on the cell membrane
- Liver and serum activity can vary with species
  - Dog and rat = low
  - Primate and guinea pigs = high
- Induced by prednisone in dogs
**Hepatic enzymes - Mouse**

**Hepatocellular Injury**
- ALT
  - 1° liver but also cardiac and testicular injury
  - 11,000% increase with mouse hepatitis virus
  - Significant elevations due to *Helicobacter hepaticus*
- AST
  - Very non-specific
  - Primarily in periportal hepatocytes

**Cholestasis**
- Alkaline phosphatase
  - Highest levels in intestine and kidney
- GGT
  - Very insensitive, normal levels very low

**Hepatic enzymes - Rats**

**Hepatocellular Injury**
- ALT
- AST
- Sorbitol Dehydrogenase (SDH)
  - Frequently the most sensitive and specific
  - Requires rapid processing
- Glutamate Dehydrogenase (GLDH)
  - Not affected by muscle

**Cholestasis**
- Alkaline phosphatase
- GGT
  - Very insensitive, normal levels very low

**Hepatic enzymes - Rabbit**

**Hepatocellular Injury**
- ALT
  - Half-life 5 hours, so changes can be missed
  - Liver activity similar to heart muscle
- Alkaline phosphatase
  - Also less specificity
- GGT
  - Very insensitive, normal levels very low

**Cholestasis**
- Hyperbilirubinemia
- ALP

**Chemistry Case - Rabbit**

- ALP – marked increase
- ALT- marked increase
- Hyperbilirubinemia
- Hyperproteinemia

**DIFFERENTIALS**
- Intestinal obstruction
- Tyzzer’s disease
- Hepatic coccidiosis
- Hepatic lipidosis

**Hepatic Coccidiosis**

*Eimeria stiedae oocyst*
Hepatic enzymes - Pig

**Hepatic cellular Injury**

- Sorbitol dehydrogenase
  - Half-life ~ 2 hours
  - Best for acute injury
- ALT
  - Not a reliable marker
  - Reduced sensitivity compared to small animals
  - Skeletal and cardiac muscle have higher activity
- AST
  - An indicator of non-specific organ damage

**Cholestasis**

- ALP
  - Decreases related to food intake
  - Some utility as a hepatic marker
- GGT

---

Hepatic Enzymes - NHP

**ALT/AST**

- Sensitive but magnitude of the elevations not as dramatic as some species (e.g. dog)
- Normal 10-50 IU/L
- With liver disease 50-500 IU/L
- Muscle Contribution
  - Often greater than liver
  - Look at the ALT/AST together with LDH and CK

<table>
<thead>
<tr>
<th>GGT</th>
<th>ALT</th>
<th>AST</th>
<th>LDH</th>
<th>CK</th>
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</thead>
<tbody>
<tr>
<td>33</td>
<td>503</td>
<td>2,340</td>
<td>16,780</td>
<td>234,900</td>
</tr>
</tbody>
</table>

**Gamma Glutamyltransferase-NHP**

- Distribution studied in macaques and baboons
  - Liver, pancreas, kidney
- More sensitive marker of biliary disease than ALP
- Higher in young animals and decrease with age
- Higher in Mauritian cynos as compared to Southeast Asian
- Can be significantly elevated with no change in ALP or bilirubin
- Persistently and progressively ↑ in amyloidosis

---

Alkaline Phosphatase - NHP

- Sensitivity as an indicator of cholestasis and biliary disease is much poorer than in many other laboratory animals
- Macaques can be as high as 1500 IU/L
- Squirrel monkeys can have high levels
- Similar to GGT, is higher in Mauritanian cynos

---

Clinical Chemistry Case: Rhesus Macaque

- 8-year-old female
- Multiparous
- In a harem breeding group
- History of chronic intermittent diarrhea
- Often cultured campylobacter
- “dirty monkey”
Day 1

- Clinical Signs
  - Very thin, 10% Dehydrated
  - Dark tarry diarrhea, melena
  - Fecal culture
    - 2+ Campylobacter
    - Susceptible to erythromycin baytril, and imipenem.

- Treatment
  - Baytril, erythromycin
  - IV albumin,
  - IV 0.9% NaCl

Clinical Signs Through Day 9

- Diarrhea continued
  - Baytril, metronidazole, imipenem
- Abortion
- Bloody vaginal discharge, mild anemia

Selected Clinical Chemistry Parameters

<table>
<thead>
<tr>
<th>Day</th>
<th>T.Bilirubin</th>
<th>GGT</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
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<tr>
<td>1</td>
<td>0.25</td>
<td>31</td>
<td>21</td>
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<td>52</td>
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<tr>
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<td>26</td>
<td>30</td>
<td>60</td>
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<tr>
<td>6</td>
<td>1.13</td>
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<td>190</td>
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<tr>
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<td>1.04</td>
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<td>223</td>
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<tr>
<td>8</td>
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<td>223</td>
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<tr>
<td>9</td>
<td>0.44</td>
<td>220</td>
<td>653</td>
<td>187</td>
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<td>1.05</td>
<td>162</td>
<td>0.44</td>
<td>220</td>
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<tr>
<td>15</td>
<td>0.13</td>
<td>1.99</td>
<td>0.28</td>
<td>203</td>
<td>193</td>
</tr>
</tbody>
</table>

Ultrasound Examination

- Uterus
  - Mildly thickened and enlarged
  - No lumen content

- Liver
  - Multifocal areas with increased hyperechogenicity.
  - Enlarged to 13 mm past caudal rib border

Clinical Pathology Changes Through Day 15

<table>
<thead>
<tr>
<th>Day</th>
<th>WBC</th>
<th>Neut</th>
<th>Lym</th>
<th>Bilirubin</th>
<th>GGT</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
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<tr>
<td>1</td>
<td>12.52</td>
<td>9.89</td>
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<td>223</td>
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</tr>
<tr>
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<td>4.35</td>
<td>0.52</td>
<td>3.44</td>
<td>0.24</td>
<td>183</td>
<td>213</td>
<td>45</td>
<td>551</td>
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<tr>
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<td>2.20</td>
<td>0.13</td>
<td>1.99</td>
<td>0.28</td>
<td>203</td>
<td>193</td>
<td>45</td>
<td>526</td>
</tr>
</tbody>
</table>

Differential Diagnosis

- Hepatitis A
- Cytomegalovirus
- Leptospirosis
- Other bacterial infection
- Drug reaction

Additional Diagnostic Tests

- Serology
  - CMV – negative
  - Hepatitis A – negative
- Bone marrow
  - Marked myeloid hypoplasia
- Liver biopsy
  - Diffuse, mild hepatocellular swelling
  - Moderate, hepatocellular vacuolar change
Imipenem

- Adverse hepatic reactions in people:
  - Can cause severe hepatic cholestasis
  - Histopathology:
    - Centrizonal bile stasis
    - Portal infiltrate rich in eosinophils
    - Cholangitis
  - Drug-induced cholestasis may be due to:
    - Direct canalicular impairment of bile secretion
    - Immunological or toxic damage to bile ducts
    - Ischemic damage to large bile ducts (sclerosing cholangitis).
  - Rarely reported to cause neutropenia

Neulasta (Pegfilgrastin)

- Human Recombinant G-CSF, PEGylated form
- Stimulates bone marrow production of myeloid cells
- Used frequently in chemotherapy patients with drug-induced neutropenia
  - 2 doses in pre-packaged syringes ordered
  - Single use syringes
  - $3500/syringe!!!!

Neutrophil Count

![Graph showing neutrophil count over time](image)

Glucocorticoids

- Mice/rats
  - Corticosterone predominates, 99% circulates bound to cortisol-binding protein and albumin
- Squirrel monkeys
  - Extremely high circulating levels
  - 1/3 of cortisol circulates unbound
  - Impaired clearance, decreased receptor affinity
  - High levels of ACTH

Muscle Biomarkers

<table>
<thead>
<tr>
<th></th>
<th>Skeletal Muscle</th>
<th>Cardiac Muscle</th>
<th>Other Tissues</th>
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</thead>
<tbody>
<tr>
<td>Creatine Kinase</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Troponin</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>B type Natriuretic Protein</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Urinary Myoglobin</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Myoglobin vs hemoglobin

- Both will cause a positive urine dipstick reaction for blood
- Both may or may not be associated with intact RBCs
- Hemoglobin quickly bound to haptoglobin and retained in serum
- Myoglobin unbound and clears quickly
- Ammonium precipitate test can confirm
Creatinine Kinase
- Rhabdomyolysis
- Capture myopathy, Exertional myopathy
- Muscular dystrophy
- Trauma
- Recumbency – large animals

Lactate Dehydrogenase
- Elevates with skeletal and cardiac muscle injury
- Very non-specific
- Mice – Lactate dehydrogenase elevating virus
- Unexplained elevations can be associated with tumors

Cardiac biomarkers
- CK Isoenzymes
- CK-MB = more cardiac specific
- Replaced by more specific assays
- Troponins (I and T)
- BNP and NT-proBNP

 Troponin
- Protein present in the thin myofilaments
- Involved in regulation of muscle contraction
- Troponin I and Troponin T
- Both good indicators of myocardial ischemia/injury
- Assays have been validated for a wide variety of species
- Interpret with caution in the face of renal disease
- Pick either I or T but do not need to run both

BNP and NT-pro
- BNP is produced by the ventricles in response to cardiomyocyte stretching
- NT-proBNP - cleaved portion of the hormone, may be less affected by degradation
- Strong indicator of heart failure
- Can be elevated by renal disease
- Species
  - Dogs – works well
  - Primates – works well
  - Rats – some assays have not cross-reacted well

Bilirubin
- Rodents
  - May be used for cholestasis
  - Heritable deficiency in bilirubin UDP-glucuronosyltransferase
    - Conjugation of bilirubin makes it water soluble so it can be cleared
    - Gunn rat – severe unconjugated hyperbilirubinemia
    - Model of Gilbert syndrome
- Rabbits
  - Total activity is normally low
  - Elevations rarely seen

Bilirubin
- Adult primates - uncommon
  - Leptospirosis
  - Hepatocellular carcinoma
  - Right sided heart failure
- Neonatal rhesus monkeys
  - Normal destruction of fetal hemoglobin
  - Immature liver
Renal Function

- BUN and creatinine
  - 50-70% of nephrons damaged
- Urinary protein
  - UPC = Urine Protein:Creatinine
    - Rodents – often elevated in rats due to chronic progressive nephropathy (CPN)
    - Often used clinically in dogs and monkeys
  - MaCr = Microalbumin:Creatinine
    - Important human measurement
    - Difficult to implement in dogs
    - Can be a good indicator of glomerular injury and GFR in primates

Urine Proteins (Rat)

- Alpha2u–globulin
  - Predominant urinary protein in adult male rat
  - Synthesized in liver under influence of androgens
  - Freely filtered by the glomerulus
  - Low levels in juvenile and senescent males
  - Minimal in females

Chronic Progressive Nephropathy

- Most severe in Sprague Dawley and Fisher 344
- Males affected most commonly
- Alpha2u–globulin - persistence in proximal tubule leads to cytotoxicity
  - Can progress to end-stage kidney
  - Can lead to tumor development
- Initial signs as early as 2 months
  - Urinary Albumin and protein ↑
  - Factors that contribute:
    - High protein diet/high caloric intake
    - Hormones – androgen, prolactin

Urine proteins

- Mouse
  - Urine concentration high in healthy mice (1.060-1.080)
  - Proteinuria is common in mice
- Mouse urinary protein (MUP) –
  - LMW lipocalin synthesized in liver
  - Structurally similar to α2u–globulin
  - Pheromone

Renal Failure in Rabbits

What is the most common cause?

*Encephalitozoon cuniculi*

- neurologic
- renal
- ocular

Urinalysis

- Rabbits
  - Urine is major route of calcium excretion – leads to cloudy, turbid urine that will become pasty if fed a high calcium diet
  - Calcium oxalate, calcium carbonate, struvite
Calcium carbonate
- Rabbits
- Horses
- Guinea pigs
- Goats

Calcium oxalate
- Can be normal in many domestic and lab animal species
- Pathogenic
  - Increased calcium excretion
  - Oxalates
  - Ethylene glycol

Urinary Biomarkers
- Proximal tubular dysfunction
  - Kidney injury molecule 1
  - Neutrophil gelatinase associated lipocalin
  - a-glutathione transferase
  - Distal tubular dysfunction
  - S-transferase Yb1
  - Distal tubular and collecting duct injury
  - Renal papillary antigen
  - Glomerular and proximal tubular
  - Albumin

Urinalysis - NHP
- Specific gravity
  - Typical range is 1.003 – 1.020
  - No defined isosthenuric range
- Urinary crystals common in macaques
- Calcium oxalate, amorphous phosphate, struvite
- Ketonuria
  - Can be seen due to negative food intake
  - Fecal contamination can cause a false positive

Macaques: Inter-female Aggression and Trauma
- Demographics
  - Middle aged female
  - No infant involved
  - Newer breeding group
- Typical Scenario
  - August – March
  - Trauma – crushing injuries
  - After hours
  - Gang up – elbows & knees

Trauma Induced Renal Failure
- Similar to "crush syndrome" in people
  - Pigment (myoglobin) induced acute tubular necrosis
  - Very common in monkeys due to the type of injury
Calcium
- Rabbits often have high circulating levels
- Absorb very efficiently from the gut independent of need or vitamin D levels
- Up to 16 mg/dl
- Can make interpretation of high calcium difficult

Lipids
- Mice
  - HDL predominant
  - No spontaneous atherosclerosis due to low levels of LDL and CETP
- Rats
  - HDL predominant
  - Overnight fast, ~30% will have hyperlipemia, but cholesterol unchanged
- Rabbits
  - Easier to induce hypercholesterolemia than the rat
  - To be effective most rodent models need to be genetically modified

Cholesterol diets
- Induce hemolytic anemia in the guinea pig
- Induce hemolytic anemia in the rat
- Proposed mechanism is decreased phospholipid in RBC membrane causing decreased deformability

Elevated Triglycerides in non-lipemic serum
- ↑ Trig usually due to ↑ in chylomicrons and VLDL which cause lipemia
- Free glycerol elevates trig without causing lipemia
- Triglyceride measurement includes glycerol
- Often seen in animals that are anorexic and on a negative energy plane
- Reported in swine
- Common in NHPs
  - Elevations as high as 2500
- Animals (humans) with diabetes

5D rats, 7 weeks, non-fasted

**SD rats, 7 weeks, non-fasted**

<table>
<thead>
<tr>
<th>Rat</th>
<th>HDL mg/dl</th>
<th>Trig mg/dl</th>
<th>Chol mg/dl</th>
<th>Glu mg/dl</th>
<th>BUN mg/dl</th>
<th>ALP IU/L</th>
<th>GGT IU/L</th>
<th>ALT IU/L</th>
<th>AST IU/L</th>
<th>CK IU/L</th>
<th>Na mmol/L</th>
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**Hydrazine administration**
- Oral gavage for 7 days
- Decreased ALT, lipids and globulins
- Hepatic lipidosis
- Inhibits pyridoxal-dependent enzymes (e.g. AST and ALT)
Serum Amyloid A

- Acute Phase Reactant
- Produced at high levels by the NHP liver
- Contributes to reactive amyloidosis
  - Common in NHP, particularly macaque
  - Frequently liver only
  - Can be systemic
  - Hamsters also have frequent amyloidosis

Evaluating Inflammation in Primates

- Neutrophilia
  - Much smaller elevations than dogs
  - Even a mild left shift is very significant
- Monocytosis
  - Small elevations can be significant
- Fibrinogen
- C-reactive Protein
- Iron/TIBC/% Saturation
  - Acute, significant decrease in serum
  - Prevalent in many NHP species

Wasting Syndromes in New World Monkeys

Marmosets

Owl Monkeys

Index Case – Adult Female Owl Monkey

Clinical signs

- Emaciated
- Ravenous
- Bright and alert
- Symmetrical, non-pruritic alopecia

Pancytopenia

- White blood cells severely decreased
- Anemic
- Low platelets

Bone Marrow Evaluation

- 18 gauge intraosseous catheter - Femur

Normal Marrow

Marrow from Wasting Animal
Myelodysplasia

Wasting syndrome to date
- Approximately 40+ animals affected
- Consistent clinical pathology findings
  - Pancytopenia
  - Profound panhypoproteinemia
  - Elevated GGT
- Vacuoles in the brain
- Large multi-nucleated giant cells in the lungs

Marmoset Wasting Syndrome
- Differences with owl monkey
  - Severe diarrhea
  - Paralysis and muscular weakness
  - Lethargy
- Attributed to:
  - Pancreatic parasites
  - Inflammatory bowel disease
  - Others

Iron overload in new world monkeys

Index case
- Squirrel monkey necropsy
  - Trauma
  - Poor body condition
  - Renal disease
  - Liver – ORANGE
  - No clinical pathology available

Normal Hepatic Iron in Primates
Liver – H&E Staining

Liver – Prussian Blue Staining

Previous Reports
- Lemurs
  - Identified in 1960s
  - 2006 – Am J Primatol, Glenn et al. - 50% incidence
- Callitrichidae
  - 1997 – Miller et al, Lab Anim Sci, common marmosets – 100% incidence
  - 2008 – Am J Primatol, Smith et al. – marmosets/tamarins – blood values correlate with liver histopathology

Prevalence
- 87 squirrel monkeys
  - 29 had serum iron >160 (33%)
- 67 owl monkeys
  - 23 had serum iron >160 (34%)
- Other findings
  - % saturation frequently approaching 100%
  - TIBC frequently mildly to markedly elevated

Associated pathology
- Concurrent disease in 50% of the animals with elevated iron
  - Weight loss, lethargy
  - Renal disease
  - Cardiac disease
  - Diarrhea
- Elevated GGT

Iron Transport
- HFE (High Fe)
  - Controls absorption from the gut
- Transferrin - binds nearly all circulating iron
  - Renders iron soluble
  - Prevents oxidative damage
  - Facilitate transport into cells
Iron Loss
- No physiologic mechanism to excrete iron, control only through absorption
- Loss through normal physiologic processes, 1-2 mg/day in
  - skin (sweat)
  - GI (stool)
  - Urine
- Females (humans and some primate species) - menses

Iron Overload in Primates and Dietary Supplementation
- Arboreal species (minimal soil contact)
- Primarily vegetarian or frugivorous eaters
- Adapted efficiently absorb iron
- Commercial diets
  - High in iron.
  - Difficult to deplete from commercial formulations

T. Cruzi in RHESUS Monkeys
- KCCMR - Texas
  - 50 adult male/female rhesus monkeys
  - Blood collected – sent to CDC for blood culture – gold standard for T. cruzi infection
  - 1/60 (positive culture)
  - 1 previous death confirmed on necropsy
- Southwest Foundation – Texas
  - 4 baboons (Williams et al, J Med Primatol, 38(2), 107-113, 2009

Trypanosomes

T. cruzi epimastigotes

Pseudocyst in the Heart
Chagas Disease: “The New HIV/AIDS of the Americas”

- PLoS Neglected Tropical Diseases
  - Baylor College of Medicine
  - 300,000 people affected in the US
  - Long incubation period
  - Hard to cure
  - End-stage heart disease
  - Other complications

Questions?