Tier One Phenotyping Project
Protocol Information

1. **Conditions**

Mice were housed in JAX® production rooms until either 8 or 16 weeks of age when sample collection took place.

2. **Health status report**

The Jackson Laboratory Diagnostics Laboratory routinely monitors and documents animal health status using statistically valid sampling procedures and standard protocols. (Sentinel animals are tested). Detailed health status reports for each animal room represented from this project are on file and available on request. See JAX® Mice Quality Control Programs or the JAX® Mice Catalog for the list of agents monitored.

3. **Housing Environment**

a. *Feed:* All strains maintained on a sterilized 6% fat diet (NIH316 formula, Purina item #5K52) from age of weaning.
b. *Water:* Adjusted with hydrochloric acid to pH 2.8-3.1 and supplemented with menadione sodium bisulfite (final concentration 0.4 mg/mL) to compensate for potential decrease in vitamin K activity upon autoclaving; water supplied in pinhole type bottles (Thoren Caging Systems, Inc. Hazelton, PA).
c. *Housing:* Thoren cages; Thoren Maxi Miser #3 cages used for some strains as either part of a PIV (pressurized individually ventilated) system or placed on conventional racking.
d. *Bedding:* Steam autoclaved white pine shavings.
e. *Photoperiod:* 14 hours on (generally covering the hours of 4:30 AM to 6:30 PM) 10 hours off.
f. *Temperature:* 18 - 24 °C
g. *Relative humidity:* ~50%.

4. **Testing period**

All testing has been done as part of an ongoing strain characterization and quality assurance program started in December 2004 and ongoing.

5. **Protocols**

a. *Body weight:*
   i. Sample collection: For 8 week data, a minimum of 10 mice were obtained at 8 weeks of age (BD +/- 3 days) from production rooms. For 16 week data, a minimum of 20 mice were obtained at weaning (3 or 4 weeks of age, depending on strain, BD +/- 1 day) from production rooms; these mice were individually identified and weighed the same day every week, from weaning until they were 16 weeks old.
   ii. Equipment: Ohaus Navigator balance, Model N12120 with internal calibration (Ohaus Corp., Pine Brook, NJ).
   iii. Consumables: 64-oz paper bucket (Sweetheart Cup Company, Owings Mills, MD).

b. *Hematology*:
   i. Sample collection: Blood (200 µL) was collected from a minimum of 10 mice via a retro-orbital bleed with a heparin-coated microhematocrit tube into a Microtainer® tube containing lyophilized K_2EDTA (BD Diagnostics, Franklin Lakes, NJ).
   ii. Equipment: ADVIA 120 Hematology System (Bayer Diagnostics Division, Tarrytown, NY, USA).

iii. Reagents and consumables: Calibrated controls (Bayer HealthCare- Diagnostics Division, Tarrytown, New York) were run before each use and were within established ranges prior to analyzing samples. (A specific list of reagents and consumables can be obtained from Sue Grindle of the Laboratory Animal Health Diagnostic Lab at The Jackson Laboratory).
iv. Sample preparation: Fresh whole blood was run on the ADVIA analyzer within hours of collection. Samples were run according to manufacturer's suggested protocol.
v. Analyzed parameters: White blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count (Plt), mean platelet volume (MPV), percentage of reticulocytes (%retic), reticulocyte hemoglobin content (CHr), reticulocytes count (#retic), percentage of neutrophils (%NEUT), percentage of lymphocytes (%LYMPH), percentage of monocytes (%MONO), percentage of eosinophils (%EOS), percentage of basophils (%BASO), neutrophil count (#NEUT), lymphocyte count (#LYMPH), monocyte count (#MONO), eosinophil count (#EOS), and basophil count (#BASO).
vi. Data calculation: Data values for Hematology parameters required no calculation.

c. *Biochemistry:*
i. Sample collection: Blood (approximately 600 µL) was collected from a minimum of 10 mice via a retro-orbital bleed immediately prior to euthanasia, using a heparin-coated microhematocrit tube, into either an Eppendorf tube or BD Microtainer® SST™ (serum separator tube). Samples were allowed to clot at room temperature for 30 minutes prior to centrifugation at 14,000 rpm for 10 minutes. The upper layer of serum was carefully transferred into a fresh 1.5 mL Eppendorf tube and frozen for later analysis.
ii. Equipment: Synchron CX5 Delta (Beckman Coulter, Inc., Fullerton, CA). A dedicated DOS-based desktop computer controls the programming of this analyzer. A dedicated printer prints the results as they are measured, and an electronic file is simultaneously transferred to a second, Windows-based computer, which stores the data files.
iii. Reagents and consumables: The following supplies and quantities were used for each sample tested: 0.5 mL Beckman Coulter Microtube™ Tubecup ("sector cup"), and a 200 µL pipette tip. For electrolyte samples, diluent consisted of either Synchron® Control Comprehensive Chemistry Control Serum Level 1 (Beckman Coulter, Inc., Fullerton, CA) or pooled mouse serum.
iv. Sample preparation: Samples for Chemistry analysis were run undiluted. Samples for Electrolyte analysis were diluted in an equal volume of diluent.
v. Analyzed parameters:
    1. *Chemistry*: Albumin, Total Protein, Blood Urea Nitrogen (BUN), Calcium, Phosphorous, Total Cholesterol, High Density Lipoprotein Cholesterol HDLC), Free Fatty Acids (FFA), Glucose, Creatine Kinase and Thyroxine (T4). Calcuim and phosphorous are included in the chemistries since that are analyzed alongside the standard analytes.
    2. *Electrolytes:* Sodium, Potassium, Chloride and Carbon Dioxide (CO_2). CO_2 is included with the electrolytes as it is analyzed at the same time as the electrolytes.
vi. Data calculation: Data values for Chemistry parameters required no calculation. Data values for Electrolyte parameters were calculated by multiplying the analyzer value by two, and then subtracting off the average of the diluent analyzer value (run in triplicate). This calculation is as follows:

(sample analyzer value x 2) - average of diluent analyzer value = reported Electrolyte value

d. *Dual Energy X-ray Absorptiometry (DEXA):*
   i. Preparation for densitometry: A minimum of 5 mice were weighed and euthanized via either cervical dislocation or CO_2 asphyxiation. Once euthanized, each mouse was placed on the specimen sticky tray (body must be within blue line on tray). The tail is placed alongside the body, the front legs are extended to the side, and the neck and spine are gently straightened.
   ii. Controls: The PIXImus is calibrated with a "phantom mouse" according to manufacturer's protocol.
   iii. Analysis: Each mouse was tested individually by placing the sticky tray on the platform under the PIXImus beam path. The X-ray takes about 5 min for full scan. The region of interest (ROI) was adjusted manually for each mouse and includes the tail and excludes the head. The ROI is defined for quality control and analysis purposes.
   iv. Data calculation: PIXImus data are from the body and tail; head area is excluded. BMD, BMC, total tissue mass, and % fat were measured. BMD and BMC adjusted for body mass and weights of lean and fat portions were derived and are available in the data set for analysis.

e. *Organ weights:*
   i. Animal preparation: A minimum of 5 mice (at either 8 or 16 weeks) were weighed and bled for hematology (see above), then immediately euthanized using CO_2 asphyxiation or cervical dislocation.
   ii. Sample collection: Immediately after euthanasia, mice were necropsied and selected organs carefully dissected for removal, placed on pre-weighed Parafilm and then weighed using a Ohaus analytical balance
   iii. Equipment: Mettler-Toledo analytical balance, Model AL54 (Mettler-Toledo, Inc., Columbus, OH)
   iv. Consumables: Parafilm M© all-purpose laboratory film (Alcan Packaging, Neenah, WI)

f. *Spleen analysis by Flow Cytometry:*
   i. Animal preparation: A minimum of 5 mice (at either 8 or 16 weeks) were weighed and bled for hematology (see above), then immediately euthanized using CO_2 asphyxiation or cervical dislocation and necropsied.
   ii. Sample collection: Immediately after euthanasia animals were necropsied and spleens were carefully dissected for removal, placed on pre-weighed Parafilm, and then weighed using a Mettler-Toledo analytical balance. A portion of each spleen was placed in a fresh Eppendorf tube and stored on ice until analyzed via Flow Cytometry.
   iii. Equipment: Mettler-Toledo analytical balance, Model AL54 (Mettler-Toledo, Inc., Columbus, OH), and either of two flow cytometers: FACSCalibur (BD Biosciences, San Jose, CA) or FACScan (BD Biosciences, San Jose, CA) with 5-color upgrade (Cytek Development, Fremont, CA).
   iv. Reagents and consumables: Parafilm M© all-purpose laboratory film (Alcan Packaging, Neenah, WI), 200 µL and 1000 µL pipette tips (USA Scientific, Ocala, FL), and 12x75 tubes (USA Scientific, Ocala, FL). Three different antibodies are mixed into one solution for both the B-cell and T-cell analysis (specific antibody cocktail recipe can be obtained from Ted Duffy or Will Schott of the Flow Cytometry group at The Jackson Laboratory).