0.0 Abstract: The Lunar PIXImus is a fully integrated densitometer using dual energy x-ray absorptiometry (DEXA) to estimate bone mineral density (BMD in gm/cm²) and body composition.

1.0 Instrument Layout:
Lunar PIXImus densitometer with its attached laptop computer, HP Desktop 930Cxi printer. With the Lunar Piximus 2.0 software.

2.0 Reagents and Expendables:
Lunar PIXImus Corporation Headquarters
726 Heartland Trail
Madison, WI 53717
Phone: 800-445-8627
Fax: 608-826-7102

Avertin (Tribromoethanol) - Anesthetic used to anesthetize animals. Use .02 ml per 1.0 gram body weight of 2% tribromoethanol solution IP prior to diagnostic testing. Avertin is provided by LAH (Laboratory Animal Health) at The Jackson Laboratory and has expiration of 2 weeks.

Phantom Mouse provided with machine.

3.0 Setup:
1. Leave the lunar machine on all time. Turn on the computer at the beginning of each day’s runs. It will automatically bring you to the main screen of the program. It is important to start in this order so that the program will communicate correctly with the Lunar equipment.
2. Click on the F6 button for the Quality Control “QC”. Put the phantom mouse in the designated spot and hit F3 (measure Quality Control Phantom).
3. Click on F3 again. It should say Quality control measurement, (setting should be 1); then click ok. The “QC” will begin to run. Once that has finished, it will say pass or fail.
4. If it says pass, click on the F5 button. This will print the “QC” for you to file in your log book. Then click on F8 to escape, this will bring you back to the main screen.
5. If it says fail you will need to start the Quality Control all over again. If it still doesn’t work you may need to run a full calibration (see section “Verify System Performance” on Page 9 of the “Piximus Series Operators Manual”). This will take approximately 90 minutes to perform. If the full calibration fails then contact the Lunar support team @ 1-800-437-1171 and follow the voice automated menu.

4.0 Run:
1. Weigh the mouse and record weight in book. Inject mouse with anesthetic (0.02 ml of Tribromoethanol 2% concentration per gram body weight of the mouse). After the anesthetic takes effect, measure the length of the mouse (nose to anus) in centimeters and record length in the book.
2. Once you have returned to the main screen of the program, you will need to click on F3 to start your measurements. This is where you will add all of the mouse's information, i.e. the weight, born date, a/n length (nose to anus length) and the histology number.
3. With the mouse asleep, put it on the sticky tray. Make sure that you stay within the blue line and
keep the mouse's back straight by aligning your two index fingers along the spine/vertebrae of the mouse's back. You will also need to position the tail to make sure that it will appear within the frame of the x-ray.
4. Put the sticky pad with the mouse on it under the beam path and begin your measurements by hitting F3.
5. While the mouse is being x-rayed, you will want to start to anesthetize the next mouse. The x-ray will take about 4-5 minutes to run the full scan. You will need to stay more than 6 feet away while the x-ray is running.
6. When the measurement is completed, you will see a colored outline of the subject with a red and green colored circle and square. You will need to click on F3 to make measurement adjustments. The green area is the one we want to delete, the red area is the one we want to keep. You will then click on F3 again to adjust ROI (region of interest). The tab key will change the color of the circle and or square. For body scans we want the square in red and the circle in green with the green circle positions over the head area. You will use your arrow keys to adjust the circle to the desired size. Holding the Control key down while doing this will enlarge or elongate the circle or square. Using the Shift key will allow you to do this rapidly. If you need to move the square to an odd angle, you can use your Page Up and Page Down keys. This will allow you to move it by rotating it left or right.
7. When desired ROI is achieved, click Enter. Then click F5 to print the scan. You will need to click F8 twice to escape; this will take you back to your main menu screen again. You are now ready to begin your next subject by repeating these steps. (After you have the desired scan, hit the Enter Key; then hit F8 twice then hit F6 to log your result. Hit F5 to print your result. After printing your scan, hit the F8 key twice to exit the scan and returned to the main screen. Hit F3 to start the next scan.)

5.0 Clean Up:
Once you have completed all your scans for the day begin clean up. Take 70% ethyl alcohol and spray and wipe everything down that you have used. Clean the outer covers and panels occasionally with a damp cloth or sponge. Use a mild detergent to remove scuffs and stains.

6.0 Data Reduction:
Place a copy of the .TXT file in the \Hlb-archive\lena\private\pixi_unprocessed folder, place a copy of the .IMG file in the \Hlb-archive\lena\private\pixi_unprocessed\IMG folder. Place copies of .TXT files in: peggy.jax.org\group\mutaprd\private\PIXIMUS\data\slg or in the subdirectory with your initials. Data placed in this subdirectory will be automatically processed into tables in Mutajax once per day. Errors found in data should be reported to the Mutajax DBA and to Randy Von Smith.

7.0 Safety:
Wear gloves and use forceps when handling subjects. Anesthetize subjects according to the Biomethodology Guidelines. When the x-ray unit is active maintain a distance of 6’ or more from Lunar PIXImus to reduce radiation exposure. Wear approved radiation exposure badge during procedure.

8.0 Time:
Set up time to get the mice and start, 1 hour. It takes 5-6 minutes to run Quality Control Phantom mouse. Each mouse takes 5 minutes prep time and 5-6 minutes to run. Clean up and data review require 1 hour. Overall production is typically 31 to 45 mice per day.

9.0 Protocol:
1. Leave the Piximus machine all the time. Turn on laptop at the start of the days runs and let it warm up about five minuets.
2. Click F6 (Quality Control) and then click F3 (Measure Quality Control Phantom). You will see Quality Control Measurement. Setting should be 1; click OK. Next, you will see Quality Control Measurement. Place Phantom Control Mouse in beam path. Click OK when phantom and tray are in place.
3. When the PIXIms starts, stand 6’ away from the machine to reduce radiation exposure. Procedure will last about 4-5 minutes. If the quality control was done correctly, you will see Last Phantom Measurement Quality Control-Passed. If it failed, it will say Last Phantom Measurement Quality Control-Failed. If it says failed, you will need to repeat the test again. If it fails a second time, shut down the entire system and start from the beginning. See 3.0 Setup. If this does not work, call the company.
4. If the **Quality Control Measurement** has passed, click **F5 (Print)** and put the sheet in the **Quality Control Log Book**. Now, you are ready to start measurements on mice.

5. Click **F8 (Escape/Cancel)** and say yes to quit; then click on the Lunar Pixi icon twice which will display the screen; then click **F3 (Measure Subject)**. You will see the Subject Info Screen. Fill in the information as directed. Click **OK** when completed.

6. You will see a picture of a mouse on the Lunar PIXImus platform. Subject is treated with 0.02 ml per 1.0 gram body weight of a 2% tribromoethanol solution IP. After the anesthesia takes effect place subject on PIXImus tray. Areas to be x-rayed must stay within the sticky pad outline. Align front and back extremities next to the mouse. Use your two index fingers to align the spine/vertebrae in a straight line and curve the tail to fit the sticky pad.

7. Click **F3 (Start Measurement)**. This takes about four to five minutes during which time you can anesthetize the next subject.

8. When measurement is complete, you will see a colored outline of the subject with red and green circle and square. Click **F3 to make measurement adjustments** and then click **F3 again to adjust ROI (region of interest)**. The area to be measured is in red, and the area not desired is in green. Remember you can switch the colors by hitting your tab key. Use the arrow keys to move the circle or square to the ROI. Use the **Control** key to enlarge or elongate the circle or square. Use the **Shift** key to move it rapidly.

9. When desired ROI is achieved, click **Enter**. Hit The F8 twice and F6 to log the result Then click **F5 to Print**. Click **F8 to Escape** and then click **F8 twice** again to go back to the main screen. You are now ready to start your next subject.

10.0 **Notes:** N/A