VO2 treadmill protocol V1.3

This is a protocol for measurement of VO2max in mice during treadmill running, which was modified from Columbus Instruments' manual and Lee-Young et al. (Wasserman) JBC, 2009; 284:23925-34.

Procedures

1. Acclimate the mice to the treadmill by running for 10 min at 10 m/min for 3 consecutive days at 9 am, prior to performing an analysis.
2. Check Drierite (should be blue). If purple, change it and SAVE purple Drierite for reuse.
3. Turn on the power (surge protector) closest to the tank to turn on the entire system.
4. Turn on the computer and login. Password is: yanlab.
5. Weigh mice. This factors into the VO2 data since units are ml/kg/hr.
6. Open **CLAMS** software.
7. Go to **Setup Experiment** tab.
    a. Calibrate oxymax
        i. Turn gas tank valve to open. Pressure should be ~10-15 psi. DO NOT adjust the adjustment valve unless the pressure is not at the target pressure.
        ii. Input certified gas values on the label of the gas tank. The values should be around 20.5% O_2 and 0.5% CO_2.
        iii. Click "Start Calibration" in the CLAMS software when all lights of the equipment are stable (ready). This process will take approximately 8 minutes. You can set Treadmill Controller parameters during this time.
    b. Select the protocol in "Treadmill Controller" - **See Below.**
    c. Select 3 cages in the **Experiment setup** tab on the right side on the screen for the oxymax to sample gases. **Important**, if 4 cages are selected, it will rotate through all 4 cages.
    d. Click "Edit" in the setup Experiment Tab.
        i. Click "Add subject". Enter a "Name" (Mouse number), "Mass" (weight), Strain (WT,AMPK KI, etc), Gender, Date of Birth
        ii. Click and drag to a specific cage or treadmill channel
8. Place mice into designated treadmill channels. Make sure the latches (top and bottom) are secure and the treadmill channels are completely sealed.
9. Turn on stimulators at 0.3 mA and 1 Hz (lights will flash).
10. Click "Data Log Location". Select location of file and name file.
11. Click "start" to start the experiment from **Setup Experiment or Run Experiment** tabs.
12. Monitor the running activities closely. Turn off the electrical stimulator when a mouse stays on the electrical grid for more than 5 seconds,.
13. Click "stop" 3 min after the last mouse stops running (allow for recording for all mice).
14. Click "Export CSV".
    a. Select cages used during the test
    b. Select the tests/experiments to export
    c. Save data file and excel files to: DESKTOP → CLAMS → DATE

VO₂max Protocol 3 Channel

Time (min)	Speed (m/min)	Incline (%)
-14	0	5
-12	5	5
0	10	5
3	13	5
7	16	5
10	19	5
13	22	5
16	25	5
19	28	5
22	31	5
25	34	5
28	37	5
31	40	5
34	43	5
37	46	5
40	49	5
43	52	5
46	55	5
49	58	5
52	61	5