

Blood Pressure Analysis Using Kent Coda 8

Before you begin:

1. If you have never used the Kent Coda 8 Blood Pressure (BP) machine, you will need to be trained by some experienced persons.
2. Mice should be ear notched at least one day before you begin measuring BP. The first mouse in the cage will be NO MARK, the second mouse will be RIGHT REAR, 3rd - LEFT REAR, 4th - RIGHT FRONT, and 5th - LEFT FRONT.

Things you need to know:

Many factors affect the accuracy of the BP measurements, including mice for the first-time measurements, low temperature, over-heating, stress, noise, inappropriate handling and so on. To improve the measurements, every member in our laboratory uses this standard protocol.

1. Our standard protocol is to measure 5 days a week. If mice are for the first time measurements, the optional approach is to do acclimation measurements prior to starting the real experimental measurements. This helps train the study mice to reduce the variability of the real BP measurements.
2. The mice should be measured the same time each day because BP varies according to the time of day (circadian rhythm).
3. For the entire study measurements (i.e. 5 days): Do not skip 2 days in a row. You may have to continue a few more days if your mice are having trouble acclimating to the machine.

Measure blood pressure:

1. Plug in the warming platforms. The Coda 8 warming platform has 3 levels of temperature control, i.e. Level 1 \approx 32 °C, Level 2 \approx 35 °C, and Level 3 \approx 38 °C. The suggested ideal temperature for the blood pressure analysis is 33-35 °C (level 2). Always use the Infrared Thermometer to check the temperature of the mouse tails. The temperature of the mouse tails should be **at least 30 °C**. If the mouse tail is too cold, BP will not be read. Conversely, if the warming platform is too hot, the mice will be stressed, and may lead to severe dehydration or even death if the temperature is too high. Therefore, watch the mouse tail temperature carefully throughout your measurements.
2. Turn on the Coda 8 BP system and the computer. Open Coda 8 program on computer (Coda 8 v3 icon).

Please test both the O-cuffs and VPR-cuffs prior to running the Coda 8.

To test the Cuffs Click Coda 8 channel 1-8

Select tested device

Device test

Select all

Test

Test results

✘ - please replace the cuff, and test the replaced cuff again.

✔ - okay.

In addition, once the machine is measuring, watch the red (O-cuffs) and blue (VPR-cuffs) lines on each graph (See the example graphs below). During the measurements, they should intersect. If they appear parallel during any measurement, the cuffs need to be replaced. Pause the machine and change the cuffs.



3. Loading mice:
 - A. Handle mice gently. Pick up mouse by the tail, place into the holder, and carefully attach the end piece as to not pinch the tail or any other body parts.



Animal Holders



VPR Cuffs



Occlusion Cuffs

- B. Thread the tail through the O-Cuff first and then through the VPR-cuff. Secure the cuffs by sliding the hose into the notch on top of the mouse holder.

- C. Place mice in their designated positions on the warming platform.
- D. Be sure to rotate mice each day in order to check the consistence of each channel. For example: On day 1, place mouse 1 in slot 1, mouse 2 in slot 2, mouse 3 in slot 3, mouse 4 in slot 4, and..... On day 2, place mouse 1 in slot 2, mouse 2 in slot 3, mouse 3 in slot 4, mouse 4 on slot 1, and.....Continue this pattern for all the mice. These rotations help improve the accuracy of the BP measurements.
4. Let mice sit in the holders with the tail cuffs on for **at least 5 minutes**. It is important to let the mice sit before measuring the BP so that they acclimate to the holders and the cuffs. *Not performing this step may result in a decrease in the percentage of the mice successfully measured.* You can also cover the holders with a blue pad if the environment is noisy or the temperature does not go up to certain level (optional). If a mouse has a wounded or too short tail, severe dermatitis, looks very sick, or is extremely irritated, do not measure the BP on that mouse. An over-irritated mouse (e.g. screaming, moving...) may also affect the measurements of the other simultaneously measured mice.
5. Enter your specimen information:
 Under Device Manager
 A. Select Coda 8 - Channels 1-8 (8)
 B. Select Use these devices
 C. Select File and choose New
 D. Under New select Experiment or Session
 The option of "Experiment": when you start a new experiment, enter the study name.
 The option of "Session": when you continue to run a same experiment, enter a session name.
 E. Type in Name of Experiment and select Researcher. Select Next.
 F. Type in Session Name. Set cycles*. Select Next.
 G. Specimen pool. Use Manage specimens to add new mice. Move mouse numbers to active specimen area. Select Next.
 H. Change cuff deflation time to 20 seconds. Select Next.
 I. Session experiment. Select Next.
 J. Experimental configuration. Select Next.
 K. Do not press the "Finish" button until all your specimens are in place and ready to be analyzed. ***This Finish button will begin blood pressure analysis immediately!*** Remember to let the mice sit for ***no less than 5 minutes*** before starting measurements to let them acclimate to the holders and cuffs. This will increase the number of successful measurements.
- Set cycles (F): You should only measure your mice for 20 cycles each run. We recommend 20 regular cycles without adding any acclimation cycle. If you don't get a measurement, do not run the mouse again at the same day.

6. Begin analysis.
 - A. Do not touch the mice, even if you are not getting any measurement. Remember, the more you handle the mouse, the more upset it will be! Never leave the room while the mice are on the platform.
 - B. Sometimes a mouse pulls its tail free from the cuffs. Pause the machine analysis and then re-thread the mouse tail. Do not touch the mouse during the running.
 - C. A run with 5 acclimation cycles and 15 regular cycles takes approximately 15 min.
7. After finishing the run, ***immediately release the mice and put them back to the cage before you do anything else.***
8. Clean mouse holders and warming platform between runs with baby wipes. Clean all areas that the mice have been in contact with. Blood, feces, and urine will upset the next group of mice. Do not use clidox, it degrades the cuffs and any plastic.

After Measuring:

1. Once the machine has finished measuring the mice, you can export the data directly to a USB stick. Export the data as an EXCEL sheet (see the following example).
2. Clean warming platform with baby wipes. Wipe the cuffs with baby wipes. Use soap and water to wash the mouse holders thoroughly with a soft brush daily. If you are measuring female mice, and the next groups to be measured are males, please use soap and water to wash the mouse holders thoroughly before you measure the male mice. Not washing the holders may lead to irritation of the male mice, and thus reducing the number of successful measurements.

***DO NOT GET THE COMPUTERS WET.**

***DO NOT PLACE ANYTHING ON TOP OF THE COMPUTERS.**

Data Analysis: Standard Parameters

- Need at least 5 actual BP readings (regular cycles) per mouse
- Standard Deviation $\leq 30\text{mmHg}$

- Systolic BP measurement must be $> 50\text{mmHg}$

An example of BP readings and analysis

Run #	Specimen Name	Specimen Description	Channel	Regular Cycle	Accepted	Diastolic	Systolic	Mean	Rate	Flow	Volume
1	A25	Mouse	1	TRUE	TRUE	115	136	122	670	12.54	39.75
2	A25	Mouse	1	TRUE	TRUE	114	142	123	676	12.46	54.25
3	A25	Mouse	1	TRUE	TRUE	121	143	128	685	11.63	54.82
4	A25	Mouse	1	TRUE	TRUE	117	144	126	617	11.26	46.54
5	A25	Mouse	1	TRUE	TRUE	122	145	129	583	16.07	53.19
6	A25	Mouse	1	TRUE	TRUE	103	132	112	603	13.91	56.07
7	A25	Mouse	1	TRUE	FALSE	118	212	149	0	5.34	55.15
8	A25	Mouse	1	TRUE	TRUE	102	145	116	697	14.11	71.83
9	A25	Mouse	1	TRUE	TRUE	109	138	118	548	17.75	71.43
10	A25	Mouse	1	TRUE	TRUE	111	143	121	471	15.38	72.83
11	A25	Mouse	1	TRUE	TRUE	124	168	138	518	16.08	96.64
12	A25	Mouse	1	TRUE	FALSE	114	124	117	0	52.11	56.81
13	A25	Mouse	1	TRUE	TRUE	111	156	126	539	16.57	87.63
14	A25	Mouse	1	TRUE	TRUE	110	143	121	530	16.06	74.85
15	A25	Mouse	1	TRUE	TRUE	113	143	123	471	19.1	85.26
16	A25	Mouse	1	TRUE	TRUE	113	147	124	613	16.96	83.12
17	A25	Mouse	1	TRUE	TRUE	98	137	111	701	17.77	64.14
18	A25	Mouse	1	TRUE	TRUE	108	139	118	558	19.29	90.84
19	A25	Mouse	1	TRUE	FALSE	115	160	130	0	16.16	94.46
20	A25	Mouse	1	TRUE	TRUE	114	129	119	666	27.82	65.51

Run #1-20: total of 20 run cycles/mouse

Specimen name: the mouse number you give, e.g. A25, here.

Channel: used channel #1 of the Koda 8 machine for this mouse BP measurements.

Regular cycles: TRUE - **acclimation** cycles

Accepted: TRUE - use the measurement; FALSE - **DO NOT** use the measurement.

For the data analysis, generally we only use the systolic BP data.

When analyze the BP data, delete all the FALSE measurements, and only use the TRUE Regular cycles, Accepted TRUE for the analysis (17 cycles in the example).

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18	A25	Mouse	1	TRUE	TRUE	108	139	118	558	19.29	90.84
20	A25	Mouse	1	TRUE	TRUE	114	129	119	666	27.82	65.51

Run #	Specimen Name	Systolic
1	A25	136
2	A25	142
3	A25	143
4	A25	144
5	A25	145
6	A25	132
8	A25	145
9	A25	138
10	A25	143
11	A25	168
13	A25	156
14	A25	143
15	A25	143
16	A25	147
17	A25	137
18	A25	139
20	A25	129
	Mean	143
	SD	9

The mean value of the accepted cycles of systolic BP (143 mmHg) represents the mean of the BP at this specific day. The Standard deviation of the mean value is 9 mmHg. Therefore, 143 mmHg is acceptable as a valid measurement.

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