**SOP for MAUI velocity trace processing in R**

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**SETUP**

1. Copy entire “LBNP” R Project folder to a location on your computer.
2. Ensure the ECG trace for all protocol sections have been extracted at 200Hz from LabChart and saved in the research drive directory 2024 LBNP Study/<id>/<visit>/ECG. Ensure the “Start of Philips Cine Loop” comments is included in the extracted section.
3. Open “LBNP\_study\_velocity\_cleaning.txt” from “data” folder. Opening in Excel will make for easier manipulation of values, as needed.
4. Change the LabChart time to seconds.
   1. Record the timing of the “Start of Philips Cine Loop” comment for each protocol in column M (“labchart\_sync\_time”) of the “LBNP\_study\_velocity\_cleaning.txt” file. Round the time down to the nearest 0.005 since data will be exported at 200Hz.
   2. For HYPOCVR, REBREATHE, and LBNPINC protocols, record the timing of the “start video” or “start camera” comment in column G. This can be rounded to the nearest second and doesn’t need to be exact. Then record the “start” and “end” time of the lowest PCO2 or end stage of LBNPINC in columns H and I (again, these can be rounded to the nearest second). These values can be eyeballed at this stage and will be fine-tuned during the processing steps. This is to highlight a section of expected low velocity where we might use a separate cleaning threshold (see PROCESSING, step 4.g below).
5. For the initial processing sweep, use similar values to previous participants for columns D, E, F, J, K which are velocity thresholds for cleaning. These values will be confirmed for each protocol/participant in the processing steps below, but a starting point is needed. I suggest a minimum threshold of 0.5 cm/s since it will help to remove noise without having a significant impact on real velocity should it drop below that level during the dicrotic notch or end-diastole.
6. Input a value of “2” in column N as a starting point for confirming the lift-off sync. This will be used (and adjusted if needed) in PROCESSING step 4.d below.
7. Save the “LBNP\_study\_velocity\_cleaning.txt” file with any changes prior to running the R script.

**PROCESSING**

1. Open “LBNP\_study\_velocity\_cleaning.txt” from data folder. Opening in Excel will make for easier manipulation of data, as needed.
2. Open R project directly from the folder.

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1. Load “VA\_imaging\_velocity\_Labchart\_sync.R” script
2. Click on Source to run. Follow instructions in Console (see following).
   1. Enter participant ID exactly as observed in choice list.
   2. Enter numerical index of visit number.
   3. Enter numerical index of protocol.

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* 1. A plot of the raw trace will be shown in the “Viewer” tab with the “rolling mean” trace and “end” of data indicated.
     1. Click on “Show in new window” to open as a tab in your web browser of choice.

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* + 1. Zoom in on the end of plot to confirm the orange “end” line is marked appropriately (i.e., does the orange line run along the lift-off signal?). Note that sometimes the lift-off signal isn’t picked up by MAUI. In this case, the orange line should be where the velocity trace drops to (near) zero.

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* + - 1. If the orange line needs shifted to the left, increase the value of the number in column “M” of the “LBNP\_study\_velocity\_cleaning.txt” file (“sync\_threshold”).
      2. If the orange line needs shifted to the right, decrease this value.
    1. If the orange line needed adjusted. Press “Esc” to quit the R-script. Save the \*.txt file with the updated “sync\_threshold “value and re-run the R-script (click on “Source”).
  1. You will then be asked to confirm the diastolic threshold for cleaning the peak velocity trace (outer envelope). A new plot is generated in the “Veiwer” tab. (Note: this threshold cleaning is just for the instantaneous signal drop out associated with MAUI and/or sample volume movement in Philips and should not be used to remove areas of complete signal loss – that must be done at the beat-by-beat analysis step.)

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* + 1. Click on “Show in new window” to open as a tab in the web browser.
    2. The purple line will show you the current threshold indicated in the “LBNP\_study\_velocity\_cleaning.txt” file. Green lines on either side show values at 5 cm/s increments from there.
    3. The R script will ask you whether you want to change the current threshold. Answer with “y” (yes) or “n” (no). If “y”, you can input the new threshold directly into R and keep going but remember to also change it in the “LBNP\_study\_velocity\_cleaning.txt” file and save (in case you need to rerun the file). If “n”, you will proceed to the next step in the R script.

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* + 1. Use the slider at the bottom of the plot to move through the acquisition, ensuring that “good” velocity traces do not drop below the purple line.
       1. If they do, adjust the “peak\_dfv” value (column E) in the “LBNP\_study\_velocity\_cleaning.txt” file, save it, and rerun the R script.
       2. You can adjust the white bars on the slider at the bottom of the plot to see more or less of the data in the large viewer.
       3. You can zoom in on the diastolic portion of the waveforms (y-axis zoom in the main plot if you wish, but this probably isn’t needed.
  1. Follow the same procedure to confirm the systolic threshold for cleaning the peak velocity trace.
     1. The current threshold is shown by the red line in the slider plot (use the same plot as for the diastolic threshold confirmation).
  2. When cleaning the HYPOCVR, REBREATHE, or LBNPINC protocols, you can ignore the blue shaded areas for the cleaning indicated above. The blue shaded areas are low PCO2 or high LBNP and the script uses a separate velocity threshold for these sections to provide more flexible cleaning. You will see in the plot below where velocity dipping near zero is normal during this phase (see red arrows) and thus cleaning will require a separate threshold or will not be possible. As mentioned above, I think 0.5 cm/s is the lowest you should go – it will provide some cleaning without removing much (if any) real data.
     1. The start and stop of the blue window is set by columns H and I (“hypocapnic\_start” and “hypocapnic\_stop”) in the “LBNP\_study\_velocity\_cleaning.txt” file. These times were “ballparked” from Labchart above (see SETUP, step 2). Based on the plot currently in the web browser, the start stop of the blue window can be fine-tuned to facilitate more exact cleaning. If the “hypocapnic” start/stop times are changed, you will need to exit the R-script (press ESC) and rerun this file after saving the “LBNP\_study\_velocity\_cleaning.txt” file with the new times.

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* 1. Follow the same procedure to confirm the diastolic threshold fore cleaning the mean velocity trace. A new plot of the intensity-weighted mean Doppler signal will be produced in the “Viewer” tab that can be expanded into your web browser.

1. When the script reaches the end, you will get a “Complete” message in the Console.
   1. The “Vewier” tab will show a plot with the raw data (red) and cleaned data (blue) at interpolated to 200Hz. You can save this interactive plot as html by choosing “Export” in the “Viewer” tab and choosing “Save as Web Page …”.

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* 1. A text file will have been created in the “output” folder with the following structure: <id>\_<visit>\_<protocol>\_VA.txt. This is a 200 Hz file with the following columns of data (units in parentheses): time (s), ecg (V), Peak\_VABV (cm/s), Mean\_VABV (cm/s), Comments. It can be loaded in LabChart for beat-by-beat extraction.

\* After loading into LabChart, a slight shift (< 1s) of the velocity waveforms compared to ECG might be required. After confirming alignment of the end of the velocity trace with the “lift off” sync comment, a shift of approx. 0.1 to 0.2 s should be sufficient to ensure the foot of the waveform is just after the R-spike (~0.1).

\*\* While not foolproof, I find this process does a really good job of cleaning the data. Certain beats (periods where the vessel was lost or where respiratory movement prevented a clean signal) will still need to be removed following the beat-by-beat extraction.