HEART RATE MEASUREMENT PROCESS

1. Fill 2 buckets with 16 L of freshwater each
    1. Remember to shut off water so pipes don't leak
2. Add MS-222 to water
    1. 11.2 mL of for every 16 L
    2. 5g MS-222 /50 mL DI water to make stock solution
    3. Pipette 5.6 mL twice

Final Concentration mg/L		Water Volume (L)	Stock solution (100 mg/ml)
50			
55			
60			
65			
70			

Formula to get volume of stock solution to get target final concentration:

volume of stock solution = (Final Concentration X Water Volume) / 100

3. Fill chiller with appropriate amount of water
    1. Check all tubes are in the right place first
    2. Continue filling chiller until water level is stabilized
4. Set temperature to on chiller to acclimation temperature of fish
    1. Notice chiller set temperature may not equal the actual temperature in apparatus
    2. Raise/lower set temperature accordingly
5. Open LabScribe application
6. Collect 4 fish
7. Soak 4 foam fish stands
8. Introduce 1 fish to bucket with MS-222 water
    1. Take fish out when they are unresponsive to tail pinch
9. Weigh fish
10. Measure length of fish
11. Place fish in foam stand and weigh down within apparatus and insert water tube into their mouth
    1. Make sure water is running through gills
12. Position electrodes on fish heart and belly (as seen in photos)
13. Weigh down foam stands with rocks
14. Fill apparatus with water
15. Replace water in chiller
16. Repeat steps 8-15 for remaining fish
17. Begin recording heart rate
    1. Rectify any "noisy" EKGs by moving electrodes
18. Calculate atropine solution (2 mL of atropine solution to 3 mL of saline solution) volume for the mass of each fish
    1. Mass x 6 = microliters of solution/1000 = mL
        1. saline solution:
            1. 7.25 g NaCl

2. 0.186 g KCl
3. 0.23 g $MgSO_4–7H_2O$
4. 0.37 g $CaCl_2–2H_2O$
5. Dissolve chemicals to 900 mL of DI water. Adjust pH to 7.8 (@10°C) and bring final volume to 1000 mL with DI water. Store in the fridge.

2. atropine solution:
    1. 0.05 g of atropine sulphate to 50 mL of saline solution
    2. store in fridge and keep away from the light, the chemical is sensitive to UV rays

19. Inject according atropine volume to each fish into intraperitoneal (abdominal) cavity
    1. Soft part of belly; be sure to not inject into muscle
    2. Make sure there are no bubbles in syringe
    3. Put syringe into safe place after use
20. Note temperature in LabScribe
21. Click “analysis” tab. Count 10 beat intervals
22. Record time seconds for 10 beat intervals
23. Return to main window on LabScribe to monitor HR
24. Calculate bpm
    1. 600 b/s / (x) seconds = bpm
25. Raise chiller 1°C (+/- difference of water temp between chiller and apparatus)
26. Set timer for 6 minutes
27. Click “analysis” tab. Count 10 beat intervals
28. Record time seconds for 10 beat intervals
29. Return to main window on LabScribe to monitor HR
30. Calculate bpm
    1. 600 b/s / (x) seconds = bpm
    2. Check fish in chamber, electrode position, gill color, mouth nozzle position, water level…
31. Repeat steps 25-30 until fish reach cardiac arrhythmia
32. Once cardiac arrhythmia occurs, note in LabScribe
33. Remove fish from apparatus and place in recovery bucket with air stone
34. Recover fish by holding over air stone (Stop calculating and counting beats, BUT DO log temperature, will come back and get the calculation after)
35. Repeat steps 32-34 for each fish as they reach arrhythmia
36. Return fish to according tank and clean up area