

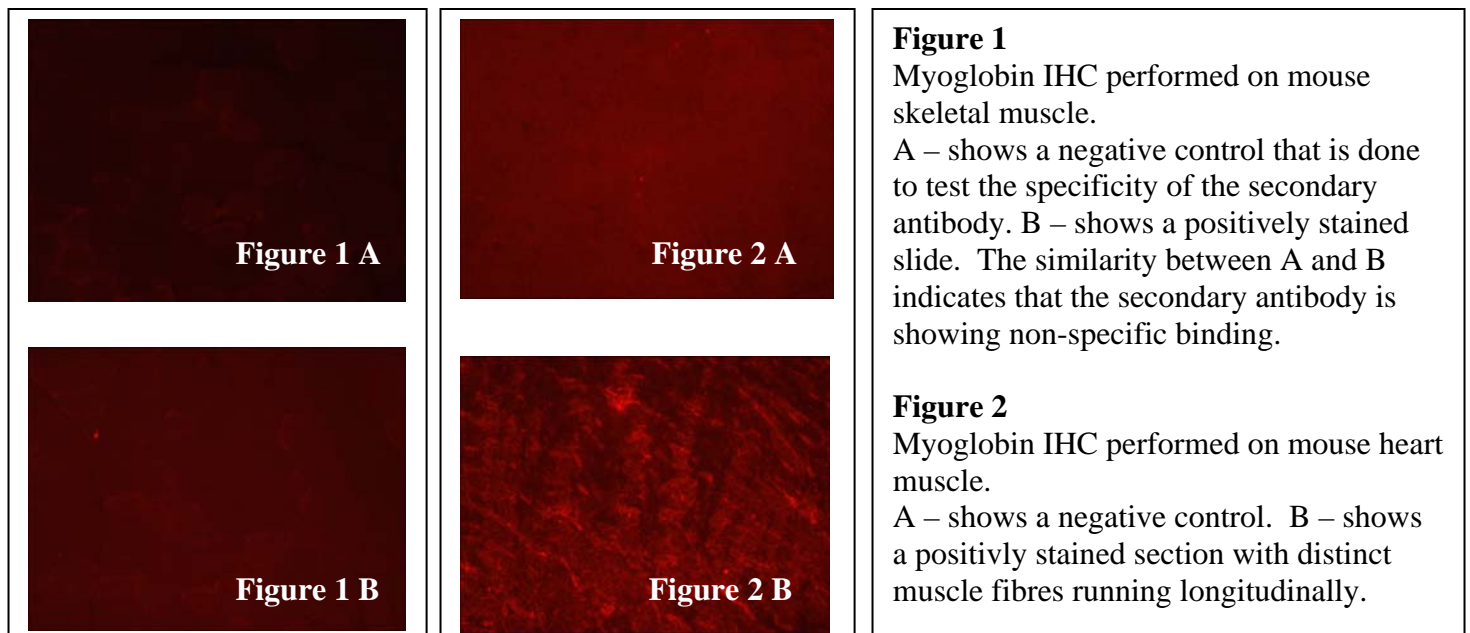
## Update #9 from the Lab Team

Sophia Kapchinsky, York University, Toronto, Canada

Hello Polar Scientists!

The final weeks of Polar Science are now upon us. This means that the Lab Team is facing its busiest season yet, while the Ice Team is slowing down and getting ready to come back home. Nevertheless, this is the perfect time for the Lab Team to complete the bulk of our research. We have now received all the seal muscle biopsies and are preparing muscle sections for staining.

In this update I will tell you about my success with myoglobin immunohistochemistry in mouse tissue and my success with Oil-Red-O staining in the Weddell seal tissue. As mentioned in my previous update (Update #6), myoglobin immunohistochemistry (IHC) was proving to be more challenging than initially expected. It appeared that the secondary antibody was showing non-specific binding. This means that the secondary antibody was binding to many different proteins in the tissue, other than myoglobin, resulting in a homogenous red image. Another problem I experienced was with the myoglobin negative control. A negative control is an experiment that is meant to test for specificity of the antibody involved. To create a negative control in IHC, I purposely applied a saline solution, called PBS instead of applying the primary antibody to one of my slides. The rest of the IHC protocol is carried out identically. In theory, since the negative control is missing the primary antibody that is meant to mark myoglobin in the tissue, the secondary antibody would have nothing to bind to. Therefore, when comparing the positively stained and negatively stained muscle, I should have an idea of what fluorescent areas are a result of specific or non-specific binding of the secondary antibody. However, as seen in *Figure 1* the intensity of the stain looks very similar for both slides. Upon consulting with Dr. Hawke, we decided to test the myoglobin antibody on heart tissue. Since it is well established that myoglobin is abundant in the muscle fibres of the heart, we decided to use it as a positive control. If the results from the positive control show little or no fluorescence then we know that that primary antibody is



not binding well to myoglobin and we may need to purchase a new antibody for the experiments. Conversely, if the results show a strong, robust color then something about the IHC protocol is hindering our results and we need to narrow down which step is causing this set back.

Figure 2 is showing the positive and negative staining of the mouse heart tissue. Several things can be noted from Figure 1 and Figure 2: (1) the positively stained section is showing bright fluorescence and striations in the muscle fibres, which is typical of the heart muscle, (2) the negatively stained section is not showing distinct muscle fibres, only a general red hue, (3) the morphology or appearance of the muscle fibres in the heart are strikingly different from those in skeletal muscle, and (4) the positive control indicates that the primary myoglobin antibody is working.

The next logical step was to go through the IHC protocol and decipher possible errors. Dr. Hawke and I decided to try a different fixation method in the hopes of rectifying the situation. Recall that fixation is a step performed after thawing the sample to make sure that the sample adheres to the microscope slide. It is possible that the fixation I was originally using was drying the muscle section and making it impossible to look for myoglobin.

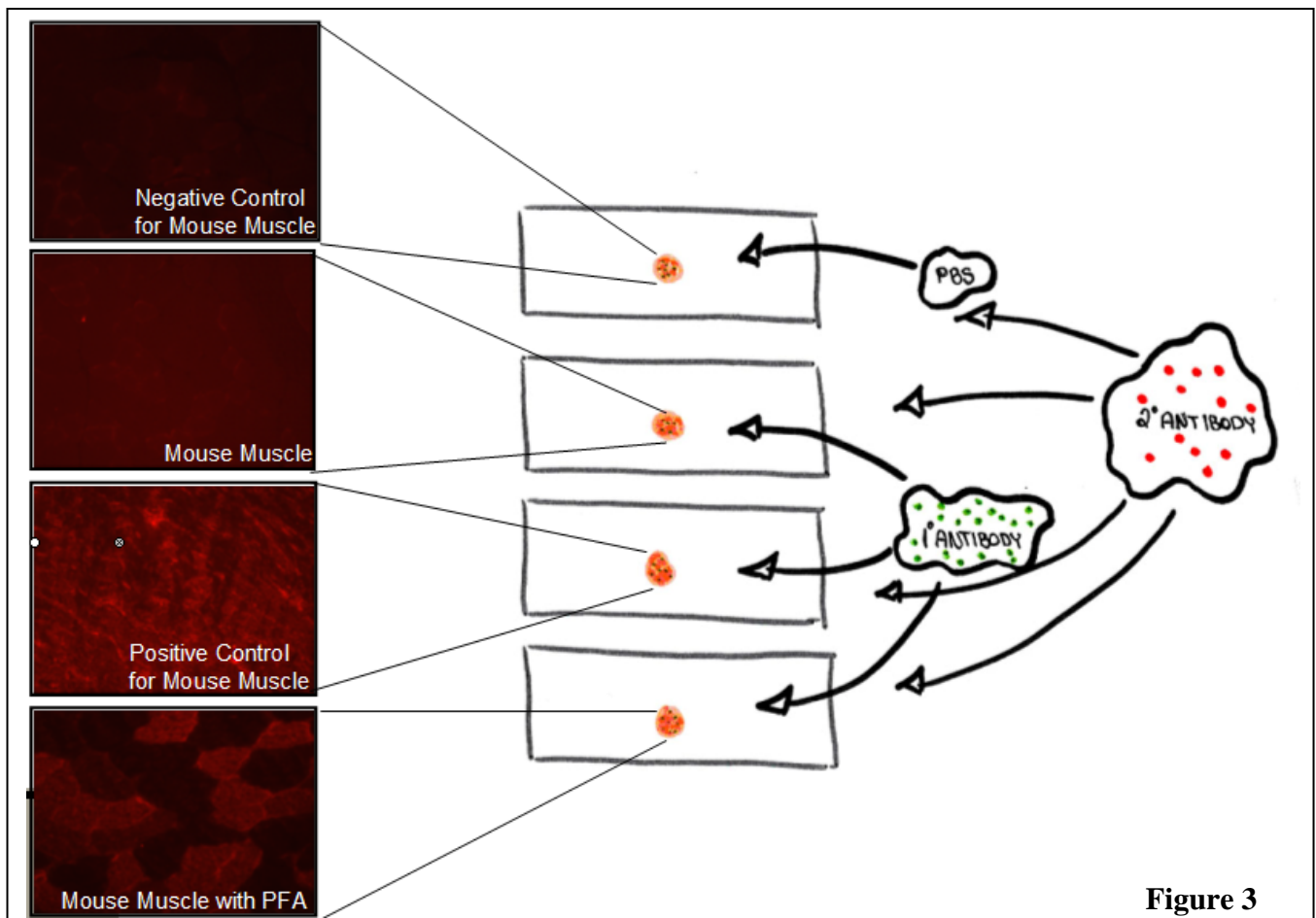


Figure 3

**Figure 3**

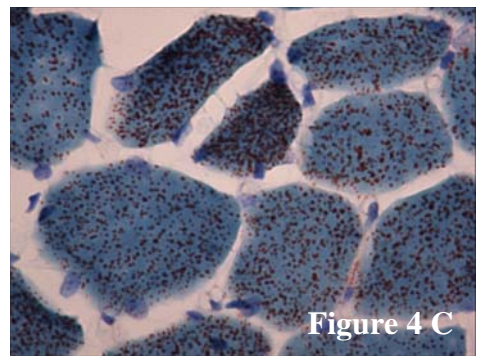
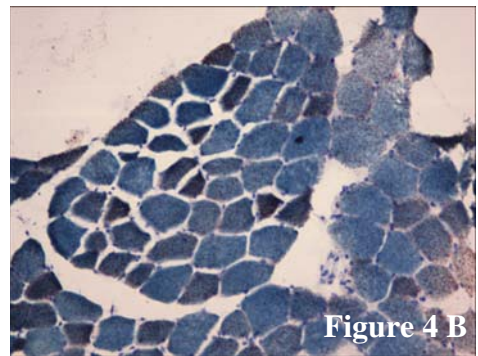
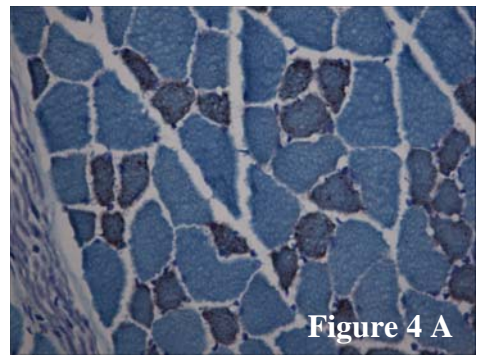
Summary of myoglobin IHC performed on mouse skeletal muscle and mouse heart muscle. There are clear differences in the final outcomes between various muscle types and protocols implemented.

*Figure 3* shows a summary of IHC done mouse skeletal muscle including a slide stained with myoglobin IHC with a paraformaldehyde (PFA) fixation. The fibres are now clearly identifiable and showing higher and lower levels of myoglobin in various areas.

The next step for myoglobin IHC is performing the procedure on Weddell seal muscle. Myoglobin IHC has never been done on Weddell seals. Therefore, it will be very interesting to see the pattern of myoglobin distribution in their muscle fibres. It is very exciting working in an area that has little or no previous information, because regardless of the results, positive or negative, it will help to further our knowledge in the area and aid other researchers in their studies.

Another area I am currently investigating is fat content in Weddell seals. As mentioned in previous updates, the Oil-Red-O protocol stains the muscle sections such that the fat droplets appear red and the muscle fibres appear blue. Jesse and I have had consistent success using this protocol on mouse tissue. Thus, the next step was to try the protocol in the seal tissue. It is always nerve-wrecking when carrying over a protocol from one animal tissue-type to another. There is no guarantee that a protocol that works in one animal, (i.e. the mouse) will show similar results in another animal (i.e. the seal). *Figure 4* shows a juvenile seal muscle section stained with Oil-Red-O. It is clear that Oil-Red-O was successful in picking out fat droplets in the seal tissue. There are very few visible differences between the mouse and seal section. This is great news that brings a sigh of relief because we can continue using this protocol without modifications.

Over the next week, I plan to: continue performing Oil-Red-O staining on the pup, juvenile and adult seal tissue; perform myoglobin IHC on the pup, juvenile and adult (hopefully with the same success as the Oil-Red-O stain) and; I will finalize the Lead ATPase protocol to get ready to use the technique on the seal sections.



#### **Figure 4**

##### **Oil-Red-O stain**

A – mouse skeletal muscle. B – juvenile Weddell seal muscle. C – juvenile Weddell seal muscle at a higher magnification. Fat droplets are clearly visible in the mouse and seal muscle. Both tissues are consistent in showing a higher fat content in the smaller fibres and a more dispersed distribution in the larger fibres

On behalf of the Lab Team, I would like to say that it has been a tremendous pleasure working with all of you over the last few months. Your questions, comments and suggestions have kept us on our toes and working hard. Thank you for participating with us on the Polar Science journey. Keep up the great work and never stop seeking explanations for your observations of your world.

Sophia Kapchinsky

Lab Team

York University