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Development of an Immunoassay to Detect Leptin, a Hormone Associated to Fat Metabolism, in American black bears

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Development of an Immunoassay to Detect Leptin, a Hormone Associated to Fat Metabolism, in American black bears

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Introduction

- Leptin has a 167 amino acid sequence (16 KDa).[1]
- Leptin is produced by adipose cells to regulate food intake, neuroendocrine function, immunity, and energy expenditure.[1,2]
- Body condition has been assessed through leptin due to positive relationship between serum concentrations and corporal adipose deposits in humans (*Homo sapiens sapiens*) and bears (*Ursus sp.*).[3-4]
- To our knowledge there are no leptin immunoassays commercially available for any bear species.
- An enzyme-linked immunosorbent assay (ELISA) uses antibodies to detect antigens (*e.g.,* leptin) and enzymes to signal antigen concentrations.[5]
- We aim to develop an ELISA to measure serum leptin in American black bears (*Ursus americanus*).

Materials and Methods

-Antibody Selection-

- Determined potential antibody cross-reactivity with bear leptin using LALIGN software[7] by comparing leptin amino acid sequences from bear, cow, mouse, and human.
- Leptin sequences from bear species (*U. thibetanus, U. maritimus, A. melanoleuca,* and partial from *U. americanus*) are identical.[6]

-Assay Optimization-

- We are assessing different concentrations of antibodies and streptavidin-HRP to find optimal assay dynamics using titration tests.

-Assay Validation-

- We will perform immunoassay validation tests (parallelism and recovery checks) to determine the ability of our ELISA to detect leptin in bear serum.[8]

- Bear vs human: amino acids 1-50 **(98.0% similar[5])**
Bear   1: mrcgplcrflwlwpylsyieavpirkvqddtktliktivtrindishtqa
Human 1: -----------y-------------v--------------------------------s

- Bear vs cow: amino acids 140-167 **(96.4% similar[5])**
Bear 140: ystevvalsrlqaalqdmlrrldlspgc
Cow 140: -------------------gs---------wq-------

Figure 1. Similarities between the Asiatic black bear (*Ursus thibetanus*) leptin amino acid sequence and those recognized by antibodies from human (*Homo sapiens)* and cow (*Bos taurus)*. Amino acids are denoted by single-letter abbreviation. Dashes are shared amino acids across species. Numbers designate the beginning of the sequence.[6]
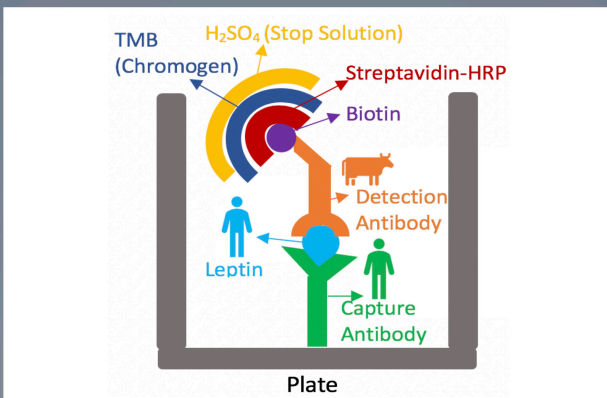
Figure 2. Diagram of proposed ELISA design.

Figure 3. A. Russo using a spectrophotometer to read a plate.
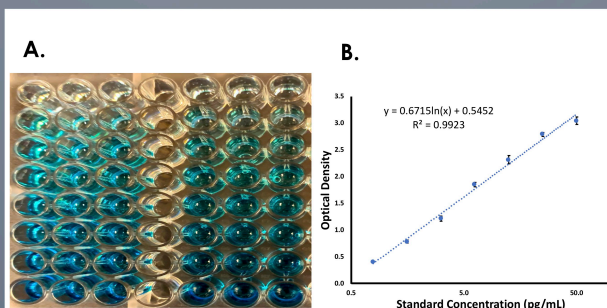
Figure 4. Titration tests. **A.** Tests in a 96-well plate: standard is absent in lighter blue wells while darker wells have high standard concentrations. **B.** Example of a proposed standard curve. Error bars are standard deviations.

Preliminary Results

-Antibody Selection-

- We selected a capture antibody targeting the C-terminal (aa 140-167) (Origene Technologies Inc. MD, USA) and a detection, biotin labeled, antibody targeting the N-terminal (aa 1-50) (Bioss Inc. MA, USA) (Fig. 1).
- Other assay components include: human leptin (Sigma-Aldrich, MO, USA) as standard, streptavidin-horseradish peroxidase (HRP) (GenScript, NJ, USA) as the enzyme, and tetramethylbenzidine (TMB) (Millipore, CA, USA) as the chromogen (Fig. 2).

-Assay Optimization-

- Titration tests are underway to determine if the selected antibody pair binds to the desired antigen. (Figs. 3 and 4).

Expected Validation Results

- Cross-reactivity between selected antibodies and bear leptin.

ELISA Applications

- This ELISA would allows us to adequately determine body condition of black bears.
- We aim to study the association between female bear body condition and reproductive parameters. This relationship could indicate potential effects of climate change and urbanization on black bear population dynamics.
- Black bears experience dramatic body changes across the active and hibernation periods. We plan to measure bear leptin to understand links between appetite and obesity with hibernation and potential applications to human medicine.

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