Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Work Plan (Main Document)

Today's Date: 13 June 2019

Date of Next Status Update Report: March 1, 2020

Date of Work Plan Approval:

Project Completion Date: 01 July 2021

Does this submission include an amendment request? __

PROJECT TITLE: Development of Advanced Diagnostic Tests for Chronic Wasting Disease

Project Manager: Peter A. Larsen

Organization: University of Minnesota

College/Department/Division: College of Veterinary Medicine, Department of Veterinary and Biomedical Sciences

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Location: Statewide

Total Project Budget: $1,804,000

Amount Spent: $0

Balance: $1,804,000

Legal Citation: M.L. 2019, First Special Session, Chapter 4, Article 2, Subd. 03t

Appropriation Language:

Diagnostic Test for Chronic Wasting Disease
$1,804,000 in fiscal year 2019 is from the trust fund to the Board of Regents of the University of Minnesota to develop diagnostic testing for chronic wasting disease that can be used to perform animal testing and environmental monitoring. This appropriation is subject to Minnesota Statutes, section 116P.10.

I. PROJECT STATEMENT: Our multi-disciplinary team will develop cutting-edge diagnostic tools for the detection of Chronic Wasting Disease (CWD) in both deer and environmental samples. CWD is a highly contagious neurological disease that is spreading throughout cervid (e.g., mule deer, white-tailed deer, elk) populations in the United States. The disease is caused by a misfolded prion protein that is spread through bodily fluids and can remain infectious in the environment for years. There is growing concern that CWD will spread widely across Minnesota, ultimately causing a significant negative economic impact to the deer hunting and farming industries. Although no human cases of CWD have been identified, conversion of human prion protein by the CWD prion has been documented in cell culture and the CDC recommends that humans avoid eating CWD contaminated meat. Despite these observations, a robust and easy-to-use diagnostic test for CWD does not exist. Available CWD diagnostics are cumbersome, time-consuming, and require significant technical expertise. For these reasons, routine testing of venison for CWD is a difficult task and it is estimated that between 15,000 and 20,000 CWD positive deer are consumed in the US annually (projected 20% annual increase). Current CWD diagnostic tests can be classified into two categories, *first-generation* "gold standard" antibody-based diagnostics (e.g., immunohistochemistry tests) and *second-generation* prion protein amplification assays. Given increasing concern that CWD will continue its spread throughout the Minnesota deer population, there is an immediate and critical need to develop advanced *third-generation* CWD diagnostics. Third-generation CWD diagnostic tests would leverage emerging microfluidic and nanotechnologies that are being developed for a variety of biomedical applications. We have assembled a multi-disciplinary team at the University of Minnesota that includes experts in prion diseases, genomics, pathogen diagnostics, and microfluidic biosensor engineering. **Project Goal:** Our team will launch research projects aimed at developing novel third-generation CWD diagnostic tools for animal testing and environmental monitoring. To accomplish this task, we will focus on the 1) identification of novel blood-based biomarkers that can identify early stages of CWD infection in live or recently harvested animals and 2) development of microfluidic technology capable of detecting CWD causing prions in a wide variety of samples collected from hunter-harvested deer, live deer, and/or the environment (e.g., feces, soil). **This project must be performed in order to 1) reduce or eliminate the consumption of CWD infected venison, 2) provide stakeholders with new tools to manage the disease, and 3) protect the rich heritage surrounding deer in the state of Minnesota.**

Our CWD diagnostic development project focuses on three main activities. First, we will identify diagnostic blood-based biomarkers (e.g., messenger and micro RNAs) that are unique to the CWD infection (led by Co-PI Pam Skinner). Preliminary data from our team and collaborators has resulted in the identification of approximately 50 RNAs that are prime candidates for CWD biomarker development. *Measurable outcome: development of CWD-specific diagnostic RNA panel that can be used with both live and recently harvested deer*. Second, we will increase CWD prion diagnostic sensitivity and accuracy by discovering miniaturized single-domain antibodies with strong binding potential to both normal cervid prion protein and infectious CWD prion protein (led by Co-PIs Peter Larsen and Davis Seelig). The small size of single-domain antibodies provides the opportunity for the development of more sensitive antibody-based CWD diagnostics. *Measurable outcome: Identification of cervid prion-specific miniature antibodies that can be used for novel antibody-based CWD diagnostics.* Third, we will modify existing protein-based biosensors within the laboratory of Dr. Sang-Hyun Oh (Co-PI) to specifically detect CWD prions extracted from both biological and environmental samples. We will also engineer novel microfluidic biosensors for CWD diagnostics (invention disclosure filed with UMN Office of Technology and Commercialization). Newly developed biosensor assays will be validated at the UMN Veterinary Diagnostic Laboratory (led by Co-PI Jeremy Schefers). *Measurable outcome: Development of novel microfluidic biosensors capable of fine-scale detection of the CWD causing prion.*

II. OVERALL PROJECT STATUS UPDATES:

First Update March 1, 2020

Second Update September 1, 2020

Third Update March 1, 2021

Fourth Update September 1, 2021

Fifth Update March 1, 2022

Final Report between project end (June 30) and August 15, 2022

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: Development of diagnostic RNA panels for CWD
Description: Blood-based biomarkers will consist of several RNAs (e.g., messenger RNAs and micro-RNAs) that are stable within blood sampled from both live and recently harvested deer. These RNAs are known to have diagnostic utility for other protein misfolding diseases (e.g., scrapie) and we anticipate the development of deer-specific RNA panels capable of identifying early stages of CWD infections. Based on our preliminary data, we have identified ~50 RNAs with strong CWD biomarker potential. Our objective is to confirm whether or not these RNAs are suitable for CWD diagnostics. To accomplish this objective, we will perform a series of RNA-seq experiments using blood and tissue samples from both CWD positive and negative white-tailed deer. Multiple RNA-seq experiments will be performed at the University of Minnesota Genomics Core resource with advanced next-generation sequencing technology. RNAs that are significantly differentially expressed (CWD vs. controls) will be selected for downstream diagnostic development. Initial diagnostic procedures will consist of targeted real-time quantitative PCR using probes specifically designed to target the differentially expressed CWD-related RNAs. These RNA probes will be validated through collaboration with the UMN Veterinary Diagnostic Laboratory.

ACTIVITY 1 ENRTF BUDGET: $505,408

Outcome	Completion Date
1. Probes designed for known differentially expressed RNAs during prion disease infection	1 December 2019
2. Discovery of novel RNAs from positive and negative controls	1 July 2020
3. Development of RNA-based panel for diagnostic validation of live and recently harvested deer	1 June 2021

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Third Update March 1, 2021

Fourth Update September 1, 2021

Fifth Update March 1, 2022

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ACTIVITY 2 Title: Single-domain Antibody (Nanobody) Development; Improvement of CWD detection assays
Description: Our second activity focuses on the discovery and diagnostic development of miniature single-domain antibodies (e.g., nanobodies) that are specific to the cervid prion protein. The small size of these novel antibodies makes them ideally suited for the development of advanced diagnostic tools. To accomplish this goal, we will collaborate with Dr. Helen Dooley of the University of Maryland. Dr. Dooley is an expert on nanobody production and we will produce nanobodies that are specific to both normal and misfolded cervid prion proteins using her model system. RNA from the nanobodies produced in the Dooley lab will be harvested, tested against the deer prion antigens, expressed in a phage or yeast display library, and purified using expressed antigen-

binding clones in recombinant proteins. These nanobodies will then be used for the improved detection of deer prion proteins in antibody-based detection assays. We will also use deer-specific nanobodies to enrich for CWD prions in diagnostic protein amplification assays. These nanobodies will be attached to nanoparticles or flow-cells for 3rd generation microfluidic diagnostic development (described in Activity 3). The outcomes of Activity 2 will result in the production of highly advanced CWD antigen-specific nanobodies with enormous diagnostic potential. It is the small physical size of these molecules that will help propel the design of 3rd generation CWD diagnostic tools.

ACTIVITY 2 ENRTF BUDGET: $676,786

Outcome	Completion Date
1. Identification of cervid prion specific single-domain antibodies (e.g., nanobodies) *that can be used for novel antibody-based CWD diagnostics*	1 May 2020
2. Antibody purification and validation of cervid prion specificity	1 December 2020
3. Development of novel prion enrichment strategies, modification of antibody-based assays	1 June 2021

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ACTIVITY 3 Title: Microfluidic biosensor development, validation at the UMN Veterinary Diagnostic Lab
Description: Our UMN Co-PI, Dr. Sang-Hyun Oh, is a world-renowned expert in the development of advanced diagnostic biosensors. Dr. Oh's laboratory specializes in the engineering and testing of microfluidic platforms using nanofabrication. We have submitted intellectual property to the UMN Office of Technology and Commercialization that provides the specifications for a microfluidic flow-cell capable of the rapid detection of CWD prions. This flow-cell will be engineered and tested within the laboratory of Dr. Oh. Immediate actions within Activity 3 include the testing of deer prion antibodies using surface plasmon resonance (SPR) technology. SPR technology uses antibodies that are anchored to a miniature gold surface. In brief, we will determine the diagnostic utility and sensitivity of SPR for the identification of deer prion proteins extracted from both biological and environmental samples. Using a microfluidic flow system, samples with deer prions will be washed over the gold flow-cell and the resulting binding will be measured using measures of light absorption. The SPR technique underlies a growing number of biomedical biosensors that are currently being used for diagnostics in human medicine. We will modify our SPR based biosensor for deer prion protein detection, including the pathogenic CWD prions. Using nanofabrication, we will design a miniaturized biosensor that is capable of amplifying and detecting CWD prions. Biosensor development will be performed in close co-ordination with the UMN Veterinary Diagnostic Laboratory. CWD samples will include known positive and known negative controls as well as blind samples (positive/negative status unknown to us). Testing of the 3rd generation assays will be performed using these controls and blinds, at the VDL, within our laboratories, and with stakeholders tasked with managing CWD in Minnesota (e.g., the Dept. of Natural Resources and Board of Animal Health). The outcome of this activity will be the engineering and testing of a microfluidic biosensor prototype, capable of detecting CWD prions in both biological and environmental samples.

ACTIVITY 3 ENRTF BUDGET: $621,806

Outcome	Completion Date
1. Antibody testing using surface plasmon resonance (SPR)	1 December 2019
2. Confirmation of optimal prion enrichment strategies using SPR and modified protein amplification assays	1 December 2020
3. Engineering, testing, and validation of microfluidic biosensor capable of fine-scale detection of the CWD causing prion.	1 June 2021

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Second Update September 1, 2020

Third Update March 1, 2021

Fourth Update September 1, 2021

Fifth Update March 1, 2022

Final Report between project end (June 30) and August 15, 2022

IV. DISSEMINATION:

Description: Milestones within each of the three main activities described herein will be made publicly available through peer-reviewed publication, presentations at scientific conferences (e.g., annual Prion meetings), and scheduled meetings with stakeholders/collaborators tasked with managing CWD in Minnesota (the Dept. of Natural Resources and the Board of Animal Health). Moreover, we are creating a website that will document our progress and that will provide outreach and educational material focused on CWD biology and diagnostics. We anticipate this website will be active in September 2019 and updates will be posted monthly as needed. Existing updates have been made on the Dept. of Veterinary and Biomedical Sciences webpage https://vetmed.umn.edu/node/9626 . We anticipate our work will result in the filing of one or several patents and, for this reason, public release of inventions will be coordinated with the University of Minnesota Office Technology and Commercialization.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

First Update March 1, 2020

Second Update September 1, 2020

Third Update March 1, 2021

Fourth Update September 1, 2021

Fifth Update March 1, 2022

Final Report between project end (June 30) and August 15, 2022

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than $5,000: To accomplish the objectives of the proposed research we must purchase equipment that will be used within our biosafety level 2 prion-rated labs. Moreover, we are establishing a biobank of CWD positive deer samples alongside negative controls. These samples, and all associated byproducts (e.g., proteins, RNA) must be stabilized at -80C. Capital expenditures include a microplate reader for RT-QuIC analyses ($30,000), two -80 freezers ($15,000 each), real-time PCR instruments (lab based and field based) for RNA biomarker development ($35,000 and $18,000, respectively), refrigerated centrifuge for protein enrichment ($20,000), an imaging system for protein detection analyses ($38,000), an Agilent bioanalyzer for measuring RNA quality ($18,000), and a nanophotometer for protein quantification ($17,000). Freezers secured using ENRTF funds will have long-term utility as they serve as the cornerstone for our cervid biobank. All other capital equipment will used within our prion research cluster throughout the lifetime of each unit.

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours for entire duration of project: 8,320	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 4

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

Enter Total Estimated Contract Personnel Hours for entire duration of project: n/a	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: n/a

VI. PROJECT PARTNERS:

A. Partners outside of project manager's organization receiving ENRTF funding

Dr. Helen Dooley; University of Maryland School of Medicine. Dr. Dooley will assist with the discovery and development of nanobodies having diagnostic utility for CWD.

B. Partners outside of project manager's organization NOT receiving ENRTF funding

Dr. Lou Cornicelli and Dr. Michelle Carstensen, MN DNR. Drs. Cornicelli and Carstensen will play important roles throughout the project and will assist not only with securing deer samples for testing, but also with the diagnostic development and validation. It is important that any diagnostic test emerging from the research discussed herein has utility for managing CWD as it spreads throughout the State.

Dr. Ed Hoover, Colorado State University. Dr. Hoover will assist by providing confirmed CWD positive and negative control samples. He will also provide cell cultures for the production of prion recombinant protein.

VII. LONG-TERM- IMPLEMENTATION AND FUNDING: We anticipate our work will result in the development of advanced diagnostic assays for the rapid detection of CWD prions in live cervids, harvested cervids, and within environmental samples. The RNA diagnostics discussed in Activity 1 (led by Dr. Skinner) will be of enormous value for both the farmed cervid industry and to detect CWD RNA signatures in recently harvested deer. The prion focused assays discussed in Activities 2 and 3 (led by Drs. Larsen, Seelig, and Oh) will provide biosensors that are capable of detecting CWD prions in a wide variety of samples. The results of our work will consist of a new class of sensative and accurate 3rd generation CWD diagnostic tools. These tools will be validated at the

UMN Veterinary Diagnostic Lab (led by Dr. Schefers) and will be shared with stakeholders tasked with managing CWD in Minnesota. After project completion, any technology that has commercial value will be routed through the UMN Office of Technology and Commercialization to identify industry partners. Given the spread of CWD throughout the USA and Canada, we anticipate that 3rd genertation CWD diagnostic tools will be of great importance as they will help to provide a real-time assesment of the CWD landscape. Such information will help stakeholders manage the disease and limit its spread.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2022

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet
D. Acquisition, Easements, and Restoration Requirements
E. Research Addendum

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Budget Spreadsheet
Legal Citation:
Project Manager: Peter Larsen
Project Title: Development of Advanced Diagnostic Tests for Chronic Wasting Disease
Organization: University of Minnesota
Project Budget: $1,804,000
Project Length and Completion Date: 2 Years 30 June 2021
Today's Date: 7 June 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 673,018		$ 673,018
PI Peter Larsen, academic and 1 month summer, $18,241 (64% salary, 36% benefits), 3% academic, 30% summer for 2 years ($36,481 total)			
Co-PI Pam Skinner, academic and 1 month summer, $21,822 (64% salary, 36% benefits), 3% academic, 30% summer for 2 years ($43,644 total)			
Co-PI Davis Seelig, academic, $14,267 (64% salary, 36% benefits), 8% academic for 2 years ($28,535 total)			
Co-PI Sang-Hyun Oh, 1 month summer, $22,377 (64% salary, 36% benefits), 30% summer for 2 years ($44,755 total)			
Post-doctoral Associate Devender Kumar, $65,282 (75.7% salary, 24.3% benefits) 100% for 2 years ($130,565 total)			
Post-doctoral Associate Oh Lab, $69,048 (75.7% salary, 24.3% benefits) 100% for 2 years ($138,097 total)			
Post-doctoral Associate Seelig and Larsen Lab, $69,048 (75.7% salary, 24.3% benefits) 100% for 2 years ($138,097 total)			
Researcher 3, Lab Manager Suzanne Stone, $56,241 (70.5% salary, 29.5% benefits) 100% for 2 years ($112,484 total)			
Total salary and benefits over life of grant $673,018			
Professional/Technical/Service Contracts			
Dr. Helen Dooley, prion-specific nanobody development	$ 80,000	$ -	$ 80,000
Equipment/Tools/Supplies			
Supplies for RNA extraction, protein purification, RT-QuIC reagents	$ 70,582		$ 70,582
Microfluidic flowcell engineering, SPR reagents, biosensor nanofabrication	$ 140,000		$ 140,000
RNA-seq library prep fees, mRNA and microRNA assays, Illumina sequencing at UMN Genomics Core	$ 142,000		$ 142,000
-20 freezer	$ 1,400		$ 1,400
Glass door refrigerator for prion lab (VWR)	$ 4,780		$ 4,780
Benchtop centrifuge	$ 4,036		$ 4,036
Prion Lab equipment, glass-ware, consumables, general western blot equipment and imaging reagents, pipettors	$ 145,184	$ -	$ 145,184
Quibit Fluorometer and reagents for RNA quantification	$ 4,000		$ 4,000
Materials and supplies for protein amplification assays, antibody-based assay development, RNA probe design and testing, and validation experiments within Veterinary Diagnostic Laboratory	$ 170,000		$ 170,000
Recombinant protein production	$ 70,000		$ 70,000
Synthetic antibody production	$ 60,000		$ 60,000
Protein analyses at UMN Proteomics Core	$ 20,000		$ 20,000
Tissue collection and preservation	$ 10,000		$ 10,000
Capital Expenditures Over $5,000			
-80 freezer for RNA sample storage	$ 15,000		$ 15,000
-80 freezer for cervid tissue storage	$ 15,000		$ 15,000
BMG Labtech Omega Microplate Reader (RT-QuIC analyses; Veterinary Diagnostic Lab)	$ 30,000		$ 30,000
BioRad CFX384 RT-PCR machine	$ 35,000		$ 35,000
MIC qPCR instrument; field deployable	$ 18,000	$ -	$ 18,000
Implen NanoPhotometer for protein quantification	$ 17,000		$ 17,000
Allegra x-30R Beckman Coulter Refrigerated Centrifuge	$ 20,000		$ 20,000
BioRad ChemiDoc MP Imaging System (western blot analyses and imaging)	$ 38,000		$ 38,000
Agilent Bioanalyzer 2100	$ 18,000		$ 18,000
Printing			
	$ -	$ -	$ -
Travel expenses in Minnesota			
Travel to attend in-state conferences to disseminate results	$ 3,000	$ -	$ 3,000
COLUMN TOTAL	$ 1,804,000	$ -	$ 1,804,000

OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		$ -	$ -	$ -
State: Rapid Agricultural Response Fund	secured	$ 259,230	$ 50,000	$ 209,230
In kind:		$ -	$ -	$ -

PAST AND CURRENT ENRTF APPROPRIATIONS	Amount legally obligated but not yet spent	Budget	Spent	Balance
Current appropriation:		$ -	$ -	$ -
Past appropriations:		$ -	$ -	$ -