2019 Project Abstract
For the Period Ending June 30, 2023

PROJECT TITLE: Development of Advanced Diagnostic Tests for Chronic Wasting Disease
PROJECT MANAGER: Peter A. Larsen
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Subd. 03t as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

APPROPRIATION AMOUNT: $1,804,000
AMOUNT SPENT: $1,804,000
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results
We invented the world's first portable 24-hour Chronic Wasting Disease (CWD) test (Minnesota-QuIC) and a 4-hour microfluidic CWD test. These tests will undergo USDA validation and will be made available to agencies tasked with controlling the spread of CWD. Our innovative CWD outreach activities and products reached over 28,000 Minnesotans.

Overall Project Outcome and Results
Chronic Wasting Disease (CWD) is a highly contagious prion disease that is spreading throughout deer populations in Minnesota. CWD threatens the health of Minnesota deer, and there is concern that CWD will cause significant negative economic impacts to all deer-related industries. A major limitation in the fight against CWD is that existing diagnostic tools are slow and complicated to use. This prevents advanced management efforts that can prevent the spread of the CWD. Thus, our team set out to invent new CWD diagnostic tools that would be faster, portable, and as good as or more accurate than existing tests. We formed a team of biologists and engineers and collaborated with state, tribal, and federal agencies. In 2020, we made a discovery that led to the world's first portable 24-hour CWD test, which we named Minnesota-QuIC (MN-QuIC). We successfully field-tested MN-QuIC in Rushford, MN in 2021 and are now working with the United States Department of Agriculture to officially validate the test. In 2021, we made another discovery that led to a rapid microfluidic test capable of detecting CWD prions in less than 4 hours. This test will also undergo USDA validation. In addition, our team brought RT-QuIC technology to the state, a highly advanced and sensitive lab-based test for CWD that can detect prions in biological and environmental samples. Using RT-QuIC, we became the first team to successfully test for CWD prions in deer muscle. We are now working to provide muscle testing to the public to help protect deer hunting activities in the state. Additionally, our team discovered biomarkers and miniature antibodies that will ultimately help fight the war against CWD through diagnostics and, potentially, therapeutics. Beyond diagnostics, we have emerged as leaders in the state regarding the science of CWD. Our CWD outreach efforts have connected with over 28,000 Minnesotans since 2019.

Project Results Use and Dissemination
Our outreach activities consisted of over 43 events, reaching an estimated 28,363 Minnesotans through both direct and virtual presentations. We developed and disseminated an array of outreach materials including: fact-sheets translated into multiple languages, websites with helpful CWD information, animations and virtual posters to help the public better understand the biology of CWD, and a webinar on the science of CWD. Our research efforts have been highlighted in over 54 media releases. We have also produced five publications in

peer-reviewed journals and 24 presentations at scientific conferences or venues, collectively advancing the science of CWD surrounding diagnostics and surveillance.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Work Plan (Final Report)

Today's Date: 29 December 2022

Date of Next Status Update Report: Final Report

Date of Work Plan Approval: 17 June 2019

Project Completion Date: 29 Dec 2023

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: $1,804,000

Balance: $0

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Subd. 03t as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

Appropriation Language:

> ***(t) 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.*

M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2023]

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 and ELISA tests) and *second-generation* protein amplification assays (e.g., PMCA and RT-QuIC). 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 1) the identification of novel blood-based biomarkers that can identify early stages of CWD infection in live or recently harvested animals and 2) the 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.

II. OVERALL PROJECT STATUS UPDATES:

First Update March 1, 2020

Our team has made great progress with respect to our overall goal of advancing diagnostic tools for CWD detection. LCCMR funds facilitated the outfitting of a new prion research laboratory within the UMN College of Veterinary Medicine. This molecular lab is approximately 900 sq ft and is entirely focused on prion biology and diagnostic development. We hired 4 staff to assist with the project activities outlined below. We have successfully performed a newly-developed CWD diagnostic test that is capable of detecting prions within both biological and environmental samples. This test is known as RT-QuIC, a prion protein amplification technique originally developed for human prion disease research. RT-QuIC functionality is essential for one component of our CWD diagnostic R&D effort. To our knowledge, and thanks to the LCCMR funds, our lab is the only lab within the state of Minnesota that has RT-QuIC functionality. We describe this development in greater detail below (Activity 3). To date, we have acknowledged LCCMR in one peer-reviewed publication (identified in Activity 3) and in 15 public presentations/seminars provided by Dr. Larsen. Our work has also received media attention and we have acknowledged LCCMR funds during our interviews and media releases from the UMN College of Veterinary Medicine. We have been making good progress on each of the three primary research activities (described below), however, our research efforts have been paused since mid-March due to the COVID-19 pandemic. The University of Minnesota placed all non-COVID related research into hibernation in March and we are anticipating resumption of research activities in June. We will keep LCCMR abreast of the situation.

Second Update September 1, 2020

The CWD diagnostic development team continues to make positive progress with respect to the goal of creating new tools for the rapid detection of CWD-causing prions. It should be noted that our research operations are

beneath 50% capacity given the ongoing pandemic. Our research labs must follow the University of Minnesota regulations with respect lab capacity, social distancing while in the lab, etc. These guidelines were established in June of 2020 and remain in place. Despite reduced operations we have made several exciting and key discoveries during the summer and fall of 2020. These discoveries have resulted in the filing of an additional invention disclosure form with UMN Technology Commercialization (relating to Activity 2, highlighted below). There are now two disclosures officially filed related to LCCMR funded research and a third will be filed in November 2020 (relating to Activities 2 and 3, highlighted below). We anticipate the filing of at least two to three provisional patents resulting from our research over the next 3 to 5 months. Regarding our recent success with RT-QuIC (previously discussed) we have routinely documented the sensitivity and specificity of the method within our main MNPRO laboratory using CWD positive and negative materials. We are working with the MN DNR on the validation of the method using tissues collected from over 500 white-tailed deer (secured from the Preston area of MN). We are completing the validation process now and anticipate our first RT-QuIC focused publication to be submitted in December / January. Additionally, we are working with the MN BAH to secure environmental samples from cervid farms that have gone CWD positive. We are collaborating with our RT-QuIC laboratory network colleagues to develop a soil-based protocol capable of detecting CWD prions in soil. We have modified our dissemination activities in light of the ongoing pandemic (described below).

AMENDMENT REQUEST 30 October 2020

Due to our reduced operational capacity during the pandemic and the impact on research progress, we are requesting a 1 year no-cost extension. All laboratories at the University of Minnesota are operating with limited staff per official university guidelines. This has impacted our ability to perform our proposed research across all Activities. Moreover, this extension is also key regarding the provisional patents that we are about to submit, as the filing of a patent results in a 1-year timeline to generate as much supporting data for the official patent filing. We have included a modified budget of our remaining funds that extends our research through 30 June 2022.

AMENDMENT REQUEST March 18 2021

We are requesting our personnel budget be increased to accommodate research staff associated with Activities 1, 2, and 3, thus accounting for our 1 year no-cost extension (requested on 30 Oct 2020). For this reason we are requesting that funds be shifted from the Equipment/Tools/Supplies budget line to personnel.

- Supplies for protein purification and RT-QuIC reagents reduced from $70,582 to $47,208
- Microfluidic biosensor nanofabrication reduced from $140,000 to $34,000
- RNA-seq library prep and Illumina sequencing reduced from $142,000 to $137,000
- Glass door refrigerator for prion lab ($4,780) eliminated
- Prion lab equipment reduced from $145,184 to $133,154
- Antibody production reduced from $60,000 to $50,000

One centrifuge listed in supplies was estimated at $4,036 but actual price was $7,410 (thus should be moved to capital equipment)

Materials and supplies for the UMN Veterinary Diagnostic Laboratory (VDL) for CWD testing was listed in the supplies line but is now moved to capital equipment expenditures. The new capital equipment line is CWD Diagnostic Equipment for Veterinary Diagnostic Lab and is increased by $5,000 to $175,000.

Several items listed in Capital Expenditures Over $5,000 are not needed and are eliminated to free up funds for personnel including:

- The BioRad PCR Machine
- MIC qPCR instrument
- Implen NanoPhotometer
- Agilent Bioanalzyer

The following Capital Expenditures differed from original estimates including

- -80 freezers for RNA and tissue storage
- BMG Labtech plate reader
- Allegra centrifuge
- BioRad ChemiDoc Imaging system

The requested changes amount to supplies being reduced by $327,810 and re-allocated to personnel ($243,750) and capital expenditures ($84,060).

Amendment Approved by LCCMR 4/26/2021

Third Update March 18, 2021

Research operations at the University of Minnesota remain impacted by the COVID-19 pandemic. As reported in the Sept 1, 2020 update, our labs remain at 50% or less capacity in order to follow UMN policies that limit the spread of SARS-CoV-2. Despite these restrictions, our research team has made significate progress in each of our defined Activities. Fourteen RNAs of diagnostic potential for CWD have been identified as a result of research related to Activity 1. Activity 2 research efforts have yielded several novel nanobodies with binding capacity to cervid prion protein and we are proceeding with a provisional patent for these nanobodies. Perhaps the most significant development pertains to research performed in Activity 3 that has yielded a significant advancement for CWD diagnostics. Our prion protein-focused team had a breakthrough development in October of 2020. This development has produced a field-deployable CWD test that can provide results in less than 24 hours. We successfully deployed and tested a prototype of the field-deployable test alongside the DNR in Rushford, MN during the second week of March 2021. This milestone is described in further detail below. Our prion research lab continues to make progress with respect to RT-QuIC-based diagnostics of CWD. We now have functional protocols for multiple tissue types (lymph nodes, tonsils, muscles, etc.) as well as sample types gatherable from live animals including blood and feces. We have recently completed a plant prion uptake experiment to investigate the environmental screening capacity of RT-QuIC and are developing protocols for the detection of CWD prions in plant tissues as well as soils. Our team has also secured a variety of environmental samples from CWD hotspots around the state, including water runoff from a recently depopulated CWD farm. These samples will be important for ongoing RT-QuIC protocol R&D.

Our efforts are yielding additional manuscripts that acknowledge the support of LCCMR. This includes a manuscript submitted in Feb 2021 (currently in review; preprint available here: https://www.biorxiv.org/content/10.1101/2021.03.03.433751v1). This manuscript provides important information regarding the utility of RT-QuIC for CWD diagnostics. Our results from this work indicate a multi-tissue sampling approach will provide a more accurate depiction of the CWD landscape. We are also submitting a manuscript focused on the detection of CWD prions in white-tailed deer muscles. To our knowledge, this will be the first manuscript to identify prions within a variety of muscles that are routinely consumed (i.e., backstrap, tenderloin, etc.). Our work in this area will lead to the development of high-throughput monitoring of venison products that will help prevent CWD prions from entering animal and human food chains. We are now working with the MN Dept of Agriculture on this issue. In sum, we continue to make progress despite the impacts of COVID-19. Our 30 Oct 2020 request for a no-cost extension is needed to further test and validate the diagnostic procedures that we have identified. Associated provisional patent submissions will require 1 year of confirmatory research to help secure full patents and navigate industry-level relationships.

AMENDMENT REQUEST December 7, 2021

We are requesting to rebudget Equipment/Tools/Supplies to provide additional funds for Personnel and Capital Expenditures. In addition, we are aligning funds within Equipment/Tools/Supplies to match anticipated spending on our final activities.

- Supplies for protein purification and RT-QuIC reagents increased by $16,956 from $47,208 to $64,164. We have expanded our RT-QuIC throughput capacity so that we can test more samples for CWD. The adjustment is needed to accommodate our expansion.
- Microfluidic biosensor nanofabrication increased $3,492 from $34,000 to $37,492 to match expense. We have made multiple discoveries concerning the microfluidic and nanotechnologies described in our previous reports to the LCCMR. The increase in microfluidic related expenses reflects these discoveries.
- RNA-seq library prep and Illumina sequencing reduced $107,527 from $137,000 to $29,473. The RNA-seq portion of the project, led by Dr. Pam Skinner, was over-budgeted. The price for RNA-seq has fallen precipitously in recent years due to technology upgrades at the UMN Genomics Center. The adjustment corrects this over-budgeting and is in line with the UMN Genomics Core prices.
- -20 freezer reduced by $9 from $1,400 to $1,391 to match expense. The freezer was $9 cheaper than envisioned.
- Prion lab equipment reduced by $38,360 from $119,154 to $80,794. The prion R&D lab is now fully outfitted with essential equipment to routinely perform LCCMR funded research. The exception is capital equipment (listed below) to increase throughput of our RT-QuIC testing.
- Quibit Fluorometer and reagents reduced by $1,003 from $4,000 to $2997 to match expense. We secured a quote that was cheaper than list price for this item.
- Recombinant protein production reduced by $27,049 from $70,000 to $42,951. Our protein production was made much more efficient by modifying purification procedures. We can now produce more amounts of recombinant protein (needed for many of our CWD diagnostic tests) at a cheaper rate.
- Synthetic Antibody production reduced $44,243 from $50,000 to $5,757. This line was over-budgeted for at the beginning of our research. The shark nanobodies being produced do not require extensive modification, thus resulting in the adjustment.
- Protein analyses at UMN Proteomics Core reduced $11,917 from $20,000 to $8,083 to match expenses. Because we increased protein production efficiency, we do not require as many purification tests from the Proteomics Core. This is reflected in the adjustment.
- Tissue collection and preservation reduced by $9,509 from $10,000 to $491 to match expense. We are receiving tissues from our DNR collaborators that are already processed and require limited steps to accession into our MNPRO tissue repository. This is reflected in the adjustment.
- Capital Expenditures increased by $69,841 to purchase Eppendorf EPMotion 5073. This machine is needed to expand our RT-QuIC and MN-QuIC testing to a 384-well plate capacity. Purchasing this equipment means that MNPRO lab would have throughput capacity of testing at least 564 deer for CWD every 24 to 48 hours. This will allow MNPRO lab to examine RT-QuIC and MN-QuIC testing capacity for state-level operations and scaling-up these tests within the UMN Veterinary Diagnostic Lab for thousands of samples.
- Travel budget of $3,000 was reduced to $0 due to COVID restrictions.
- Personnel increased by $152,328 from $930,768 to $1,083,096. We required additional funds to support personnel due to the COVID-related extension of the research.

Amendment Approved by LCCMR 2/2/2022

Fourth Update December 7, 2021

Our CWD diagnostic development team continues to make progress on advancing diagnostic tools for the more rapid and sensitive detection of CWD prions. We have internally validated the RT-QuIC testing platform described in our previous updates. Results of our validation show that specificity and sensitivity of RT-QuIC is as good or better than the traditional ELISA and IHC testing platforms when using deer tissues (i.e., lymph nodes, brain, obex, tonsils). Our team is preparing a publication now that describes the RT-QuIC validation process. Our

field-deployable MN-QuIC test (described in our previous update; developed by our team) is also undergoing internal validation. Thus far, MN-QuIC results are 100% accurate with RT-QuIC, ELISA, and IHC when using the same tissues. We have submitted a publication that describes the MN-QuIC test as well as two provisional patents on the technology underlying the test (preprint available below in Dissemination section). Our research efforts over the summer of 2021 have yielded several important discoveries that we believe can be leveraged to produce prion detection assays that produce results within approximately 4 hours. The supporting experiments are described in Activity 3. Moreover, the technology involved has a very small foot-print and our functional prototype is approximately the size of a cell phone. If our validation process yields results for this ultra-rapid test that are similar to our MN-QuIC assay, then the implication is that CWD testing can be performed on-site and with results in less than 4 hours. Regarding Activity 1, Dr. Skinner's lab is still investigating the diagnostic potential for deer RNA's. There are 14 promising RNA's that remain to be validated. For Activity 2, we have now identified multiple shark nanobodies that bind to the mammalian prion protein. These nanobodies have multiple utilities including diagnostic potential and could also lead to therapeutic research for blocking CWD prions from spreading within an animal. We are filing a patent on these nanobodies.

Despite our advancements, research continues to be impacted by the COVID-19 pandemic. Impacts include the delayed shipment and overall availability of laboratory supplies and molecular reagents needed to conduct our R&D research. Delays for items range from two weeks up to 3 months. We anticipate these delays to continue into 2022. The pandemic continues to impact faculty, staff and students as illnesses require COVID testing and self-isolation until results are secured.

Fifth Update March 1, 2022:
We continue to make progress for each of our activities and anticipate successful completion of the project in 2022. Regarding Activity 1: Dr. Skinner's team continues to make progress on identifying RNA biomarkers for CWD that can be used with biological fluids and/or tissue samples. Her team has submitted a manuscript to BMC Genomics that describes RNA expression levels in deer brain tissue samples. For Activity 2: We have confirmed the six shark nanobodies have binding affinity for deer prion protein. These nanobodies are now being used with western blotting methods in the research laboratory. A provisional patent will likely be filed in late 2022 or early 2023. With respect to Activity 3: We have completed the internal validation of the 24-hour MN-QuIC test and results show that it has a diagnostic sensitivity of 95.7% and specificity of 100%. This result is exciting because it shows the MN-QuIC test is as good as, or better than, the current ELISA and/or IHC tests for CWD. We have incorporated these results into our manuscript and will now submit for publication to Scientific Reports. Regarding our microfluidic testing device, we have fine-tuned the platform such that multiple prototypes can be easily produced. Additional tests continue to show that it can identify CWD positive tissues in less than 4 hours of runtime and we are not seeing evidence of false-positive results when using CWD negative tissues. We believe that the microfluidic device will provide a pathway to a deer-side test that can be used by state or federal agencies to better control the disease.

Update as of June 30, 2022:
Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:
We are excited to report that we have achieved major milestones for each of our proposed activities, listed below. We provide a brief summary here, with more details appearing below. For Activity 1, Dr. Pamela Skinner's team has published a manuscript that reports the identification of RNAs which can be leveraged for CWD diagnostics. RNA biomarkers have long been hypothesized to be potential biomarkers for CWD and the research completed by Dr. Skinner and colleagues help establish the biomarker potential of select RNA species. Nevertheless, lessons learned during the course of our research regarding RNA stability and difficulty in obtaining samples needed for CWD testing indicate that RNA will have limited potential as a CWD diagnostic for state, tribal, and federal agencies wanting to test wild deer. The advancements made in Activity 3 regarding

next-generation CWD diagnostic tools are likely superior to RNA-based biomarkers. For Activity 2, we have confirmed that the nanobodies generated effectively bind to deer prion protein, thus opening the door to future diagnostic and therapeutic advancements for CWD. The therapeutic potential for the nanobodies that we have identified cannot be understated, as they might serve as a unique method to combat CWD through innovative methods. We are determining the exact binding location between white-tailed deer prions and the nanobodies we have generated. This information will be leveraged for diagnostic potential but will also inform future therapeutic research. For Activity 3, our team is submitting our 24-hr field-deployable test, named Minnesota-QuIC or MN-QuIC, to the USDA for official validation. The USDA process for validation typically takes 1 to 2 years. Our internal lab validation process continues to be very successful for MN-QuIC, we have performed hundreds of confirmatory tests and the assay is exhibiting exceptional specificity and sensitivity. Our work on MN-QuIC has now been published in a peer-review journal (highlighted below). In addition to MN-QuIC, our team led by Dr. Sang-Hyun Oh has developed a portable CWD diagnostic tool that provides reproducible and robust results in less than 4 hours. We have named this new tool Micro-QuIC, it is currently a prototype and we anticipate future refinements into a commercializable product, representing what we hope will be the world's first deer-side test.

Overall Project Outcomes and Results

Chronic Wasting Disease is a highly contagious prion disease that is spreading throughout deer populations in Minnesota. CWD threatens the health of Minnesota deer and there is concern that CWD will cause significant negative economic impacts to all deer-related industries. A major limitation in the fight against CWD is that existing diagnostic tools are slow and complicated to use. This prevents advanced management efforts that can prevent the spread of the CWD. Thus, our team set out to invent new CWD diagnostic tools that would be faster, portable, and as good as or more accurate than existing tests. We formed a team of biologists and engineers and collaborated with state, tribal, and federal agencies. In 2020, we made a discovery that led to the world's first portable 24-hour CWD test, which we named Minnesota-QuIC (MN-QuIC). We successfully field-tested MN-QuIC in Rushford, MN in 2021 and are now working with the USDA to officially validate the test. In 2021, we made another discovery that led to a rapid microfluidic test capable of detecting CWD prions in less than 4 hours. This test will also undergo USDA validation. In addition, our team brought RT-QuIC technology to the state, a highly advanced and sensitive lab-based test for CWD that can detect prions in biological and environmental samples. Using RT-QuIC, we became the first team to successfully test for CWD prions in deer muscle. We are now working to provide muscle testing to the public to help protect deer hunting activities in the state. Additionally, our team discovered biomarkers and miniature antibodies that will ultimately help fight the war against CWD through diagnostics and, potentially, therapeutics. Beyond diagnostics, we have emerged as leaders in the state regarding the science of CWD. Our CWD outreach efforts have connected with over 28,000 Minnesotans since 2019.

III. PROJECT ACTIVITIES AND OUTCOMES:

Our CWD diagnostic development project focuses on three main activities. First, we will identify diagnostic blood-based RNA species that are unique to the CWD infection. Preliminary data from our team and collaborators has resulted in the identification of approximately 50 RNA species 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. 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. *Measurable outcome: Development of novel microfluidic biosensors capable of fine-scale detection of the CWD causing prion.*

ACTIVITY 1 Title: Development of diagnostic RNA panels for CWD

Description: Blood-based biomarkers will consist of several RNA species (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 in sheep) and we anticipate the development of deer-specific RNA panels capable of identifying presymptomatic stages of CWD infection. 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. We have secured both CWD positive and negative samples from our collaborators (MN Department of Natural Resources and Colorado State University). Multiple RNA-seq experiments will be performed at the University of Minnesota Genomics Core resource with Illumina sequencing technology. RNA species (e.g., mRNAs and microRNAs) 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. Future diagnostic development will center on the development of microarray chips with probes specific to the optimized CWD diagnostic RNAs. These microarray chips can be utilized with portable RNA biosensors developed at UMN.

ACTIVITY 1 ENRTF BUDGET: $505,408 (includes personnel salary and fringe)

Outcome	Completion Date
1. Probes designed for known differentially expressed RNAs during prion disease infection	1 December 2019
2. Discovery of novel RNA species 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 2022

First Update March 1, 2020

Activity 1 research efforts are led by Co-PI Dr. Pamela Skinner. In summer of 2019, Dr. Skinner established key collaborations with respect to the development of RNA biomarkers for blood-based CWD diagnostics. These include Dr. Stephanie Booth (Public Health Agency of Canada; CWD biomarker expert), Dr. Allen Herbst (Centre for Prions and Protein Folding Diseases, Univ of Alberta; CWD biomarker expert), and Dr. Nick Haley (Midwestern University; expert on CWD prion detection in bodily fluids). Dr. Skinner secured USDA permits to import CWD-infected tissues from within the USA and focused on securing CWD positive and negative tissues appropriate for RNA-based diagnostic development efforts. With respect to the outcomes identified in Activity 1, the Skinner lab has developed real-time quantitative RT-PCR assays for 10 genes that are potential biomarkers for early CWD detection in blood (Outcome 1). The discovery of novel RNA species from positive and negative control samples (Outcome 2) will progress over the summer depending on lab activities approved by the University during the ongoing pandemic. We have secured the necessary tissues for Dr. Skinner to accomplish Outcome 2 and we anticipate the discovery of blood-based biomarkers after successful RNA sequencing of these samples by the University of Minnesota Genomics core facility.

Second Update September 1, 2020

Activity 1 research efforts are led by Co-PI Dr. Pamela Skinner. Dr. Skinner continues to make progress regarding the identification of important RNA biomarkers that are indicative of an active CWD infection. Her team has generated PCR results showing differentially expressed RNAs of interest within CWD positive and not-detected samples. Dr. Skinner is collaborating with a large team of experts (identified above) to secure additional high-quality samples wherein RNAs are intact and available for analyses. Supporting data for Outcomes 1, 2, and 3 continues to be generated with promising results based on Dr. Skinner's previous work. As with our prion-focused team, the RNA biomarker discovery team has also experienced reduced operations given the ongoing

pandemic. Nevertheless, we anticipate the production of high-quality RNA-seq data by the UMN Genomics core facility in the coming months. These data will help to identify additional RNA biomarkers that will be included in a white-tailed deer (WTD) CWD biomarker panel. Finally, the current WTD genome assembly available to the scientific research community is lacking and of very poor quality. This aspect of the WTD genome prevents key insights into RNA biomarker discovery. For this reason, the CWD diagnostic development team will work together to produce a high-quality WTD genome assembly (through collaboration with the UMN Genomics core) in the coming months.

Third Update March 18, 2021

Co-PI Dr. Pamela Skinner's team has successfully identified 14 RNAs with biomarker potential for CWD. These biomarkers are genes that show differential expression in lymph nodes from CWD positive animals. The differential expression of the genes were first identified by RNAseq analysis (performed by collaborator Dr. Alon Herbst) and confirmed using RT-PCRF by the Skinner lab at the UMN in a cohort of 4 CWD+ and 4 CWD negative white tail deer. The identification of these 14 RNAs is important because they can be used to develop a PCR or micro-array based panel that can help to identify CWD positive animals. Dr. Skinner is also leading an effort to sequence and annotate the white-tailed deer (WTD) genome. The current WTD genome assembly is of poor quality with annotations lacking. This hurdle prevents insights into the genetics and genomics of CWD in WTD. Our teams worked together, with USDA collaborators, to secure and preserve a variety of tissues from a wild WTD from Minnesota (Ramsey Co) specifically for the improvement of the WTD genome. This resource will be a major contribution to the field and will help to develop a wide range of cervid-related CWD research projects, especially those pertaining to Activity 1 herein.

Fourth Update September 1, 2021

The RNAs identified by Dr. Pamela Skinner continue to show promise for predicting CWD status. It is possible that the RNAs could be leveraged to develop a micro-array panel capable of predicting CWD using a blood sample. The process for such a test would be to secure blood from a particular cervid, extract RNA, and then measure expression levels across the specific RNAs of interest. If accurate, the approach could be used as an independent measure to examine CWD status, alongside prion-focused tests like ELISA, IHC, RT-QuIC, and MN-QuIC. We anticipate the findings of this research will be summarized in a publication in 2022. Dr. Skinner is also leading an effort to re-sequence the white-tailed deer genome in order to develop better genomic resources for fighting the war on CWD. Once assembled, the genome would be a fantastic resource for researchers exploring the genetics of CWD. We also anticipate the white-tailed deer genome assembly paper will be submitted for publication in 2022.

Fifth Update March 1, 2022

Dr. Skinner's research team continues to work on the assembly and annotation of the white-tailed deer genome. This resource will facilitate downstream identification and validation of RNA-biomarkers for diagnosing CWD using biological samples. Her research team submitted a publication to BMC Genomics that identifies RNA species that have potential utility as CWD biomarkers. The paper reports gene expression changes that correspond to CWD infection, with 255 genes deregulated by CWD and 197 upregulated. These data will help to better understand the impacts of CWD on deer health and might prove useful for future biomarkers using tissue samples.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Dr. Pamela Skinner has led the effort to identify RNA biomarkers that can be leveraged for CWD diagnostics. Her research team has published a manuscript entitled "Neural transcriptomic signature of chronic wasting disease in white-tailed deer" appearing in the peer-reviewed journal BMC Genomics. This work reports the identification of 255 genes whose RNA expression was significantly impacted by CWD. The team performed quantitative PCR methods in CWD positive deer that confirmed their findings. Dr. Skinner is completing work on a white-tailed deer genome assembly that would facilitate future RNA diagnostic work. Mammalian genome assembly methods are time consuming and we anticipate her work to be completed in 2023. In addition to the genome that Dr. Skinner is working on, several deer genome assemblies have been released over the past year, thus serving as resources for developing RNA-based diagnostic panels. Although the 255 genes that Skinner's team identified represent an important resource for understanding CWD biology, the advancements made for portable real-time diagnostics (described below) are likely superior to an RNA-based biomarker panel. This is because our research over the past 3 years has shown that high quality RNA is difficult to obtain from both wild and farmed deer. Nevertheless, it is possible that future PCR technologies might be leveraged to perform RNA-based diagnostics in a more timely and field-deployable manner. The 255 genes that the RNA team has identified represent an important discovery.

Final Report Activity:
Dr. Pamela Skinner's research team identified multiple RNAs that have the potential to serve as biomarkers for CWD. Validating the target RNAs continues and will require a variety of deer tissues and biological samples to confirm effectiveness for CWD diagnostics. Her effort to sequence and annotate the white-tailed deer genome is ongoing. Several white-tailed deer genome assemblies have been released by other teams in the past few years and these will complement the genome assembly that her team is working on. The research outputs by Dr. Skinner's team provide a potential panel of RNA targets that will help to provide a better understanding of the biology of CWD and it is possible that specific genes with up- or down-regulated expression levels will be useful as biomarkers or therapeutic targets. When considering diagnostic applications that have been identified as optimal by state, federal, and tribal agencies as well as the public, the utility of RNA biomarkers remains limited. This is because it is difficult to acquire biological samples for deer in both wild and farmed settings that can be used for rapid confirmation of CWD using RNA techniques. Nevertheless, the research output by Activity 1 has identified gene targets that will serve as the basis for future CWD research efforts.

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 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. Single-domain nanobodies are typically produced from mammalian species such as camels and llamas. However, these mammals have prion proteins that are similar to the cervid prion protein, a genomic feature that limits antibody development for our protein target. For this reason, we must leverage other species that are being used for the discovery of advanced single-domain antibodies with diagnostic utility. To accomplish this goal, we will collaborate with Dr. Helen Dooley of the University of Maryland. Dr. Dooley is an expert on nanobody production using a shark model system. The absence of a recognizable prion protein in the shark model will result in the production of robust nanobody immune response to a cervid prion protein challenge. We will produce nanobodies that are specific to both normal and misfolded cervid prion proteins using the shark model. RNA from these nanobodies will be harvested, inserted into a display vector, panned against the cervid 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 cervid prion proteins in antibody-based detection assays (e.g., IHC and ELISA). We will also use cervid-specific nanobodies to enrich for CWD prions in protein amplification assays (e.g., PMCA and RT-QuIC). These molecules 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 (includes personnel salary and fringe)

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. Generation of supporting data for patents filed relating to Activity 2 research.	1 June 2022

First Update March 1, 2020

We have launched the nanobody discovery element of our research. This portion of our project consists of an essential collaboration with Dr. Helen Dooley at the University of Maryland. Dr. Dooley's lab produces nanobodies using a nurse shark model. We have secured two nurse sharks available for our CWD diagnostic development research and these are housed within U of Maryland facilities. The nurse shark immune system produces extremely small antibodies (nanobodies) that are of great utility for next-generation diagnostic tool development. The service contract arranged by the University of Maryland and University of Minnesota took approximately 6 months to negotiate by university lawyers. Once approved by the two universities (Feb 2020), we began the challenge experiment with Dr. Dooley in line with Outcome 1 described above. This delay combined with the COVID pandemic has slowed the production of cervid specific nanobodies. Dr. Dooley's lab is operational now and we have begun the process of nanobody production. We anticipate successful completion of Outcome 1 in Fall 2020 and should remain on track for completion of Outcome 2 by December 2020. We have secured non-cervid related nanobodies from Dr. Dooley in order to test our microfluidic methods and prepare for the cervid-specific nanobodies that will have diagnostic potential.

Second Update September 1, 2020

We have successfully produced and identified shark nanobodies with high binding affinity for mammalian prion protein. These nanobodies are currently being functionalized with usage in our main MNPRO prion research lab. This line of research has much promise and will be the subject of intense effort in the coming weeks and months (Outcome 3). Given the success of our efforts, we are expanding the number of prion targets for ongoing and future shark-based experiments (Outcome 2). These additional targets have great potential regarding the identification of CWD strain variants. Activities related to Outcome 3 are ongoing. We are actively working with an industrial partner to identify mechanisms to scale-up production and incorporate our findings into low-cost diagnostic tools (with assistance with UMN Technology Commercialization).

Third Update March 18, 2021

The nanobodies identified in our previous update have been tested against the white-tailed deer prion protein and we have confirmed their binding capacity. We are now functionalizing these nanobodies for a number of diagnostic applications to be used with Activity 3 and for the development of newly conceived prion diagnostic applications. We are working with the UMN OTC to complete the provisional patent application that defines the amino acid and nucleotide sequence for these nanobodies. Additionally, we have launched a shark-challenge experiment in order to identify nanobodies having binding potential for misfolded prion proteins. If successful, this experiment will lead to the design of an entirely new class of nanobody-based CWD surveillance tools.

Fourth Update September 1, 2021

We have made great progress with the production of shark nanobodies with binding affinity to the mammal prion protein. We have now documented binding capacity of six nanobodies to our prion targets. This finding opens the door to several exciting research opportunities not only for CWD diagnostics, but also for CWD therapeutics. The small size of the nanobody allows for the development of hand-held lateral flow tests and they can cross of the blood-brain barrier. We will formally submit a patent on the six shark nanobodies in

December 2021. We have identified a commercial partner who is testing shark nanobodies for lateral flow assays. We have also performed the final inoculation of the sharks with the misfolded form of the prion protein. We anticipate the recovery of nanobodies specific to the misfolded form to occur in early 2022. If accurate, we anticipate this approach will yield ultra-sensitive and deer-side diagnostic tools as well as novel therapeutic approaches to CWD. This is one of the most exciting lines of research that MNPRO has launched, and we will be sharing more on this avenue of work in the coming months.

Fifth Update March 1, 2022
We are now using the six shark nanobodies with western blotting techniques for detecting deer prion protein in the laboratory. This means that the shark nanobodies are useful for routine lab research as well as for future diagnostic and therapeutic applications for CWD. Our collaborator, Dr. Helen Dooley, is experiencing limited availability of supplies for her laboratory due to the ongoing COVID-19 pandemic. This is slowing some of our work focused on the shark nanobodies. We anticipate these delays to be resolved in the summer of 2022. For this reason, we are delaying the submission of the provisional patent until the completion of a last round of binding experiments.

Update as of June 30, 2022:
Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:
We have confirmed that the 6 shark nanobodies produced during the course of our research bind to mammalian prion protein, including white-tailed deer. This result is incredibly important because the latest research surrounding nanobodies shows very promising potential of nanobodies for neurodegenerative disease therapeutics. Although the shark nanobodies have clear diagnostic potential, the original intent of the Activity, the therapeutic potential might represent an enormous step forward for fighting CWD. We believe it is possible to refine the shark nanobodies to interact with prions in such a way as to elicit an immune response in deer that might destroy misfolded prions. There is much future work that would need to be done to explore that potential, however, recent data from humans show that it is possible. Regarding the diagnostic potential for the nanobodies, our team is developing a real-time PCR assay that leverages the binding location of the nanobodies to CWD prions. This 3D binding orientation is followed by a RT-PCR assay (that can be performed using field-deployable equipment as well as high-throughput laboratory approaches) which produces a positive or negative result. If successful, this test could be the most sensitive CWD test ever produced because the method would be able to detect miniscule amounts of CWD prions.

Final Report Activity:
In Activity 2 we set out to accomplish a highly novel and innovative goal of leveraging the shark adaptive immune system to produce tiny antibodies (nanobodies) that have binding affinity to deer prion protein. The reason we focused on this line of research was because these tiny nanobodies have emerged as important molecules for neurodegenerative disease diagnostics and therapeutics. We successfully challenged nurse sharks with mammalian prion protein and were able to identify six shark nanobodies that can bind to the white-tailed deer prion protein. This discovery is important because we believe these nanobodies could lead to an entirely new class of diagnostic tools for CWD and might even lead to innovative therapeutic opportunities for fighting the disease. We are now mass producing the nanobodies and will continue our diagnostic research on these novel nanobodies through Activity 2 within our recently awarded LCCMR proposal entitled “Establishing a Center for Prion Research and Outreach”.

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 cervid prion antibodies (currently available and newly developed by our team) using surface plasmon resonance (SPR) technology. In brief, we will determine the diagnostic utility and sensitivity of SPR for the identification of cervid prion proteins extracted from both biological and environmental samples. SPR technology uses antibodies that are anchored to a miniature gold surface. Using a microfluidic flow system, samples with cervid prions will be washed over the gold flow-cell and the resulting binding will be measured using light absorption indices. The SPR technique underlies a growing number of biomedical biosensors that are currently being used for diagnostics in human medicine. We will modify an SPR based biosensor for cervid prion protein detection, including malformed pathogenic CWD prions. Using nanofabrication, we will design a miniaturized flow-cell that is capable of amplifying and detecting CWD prions. To accomplish this, we will use prion enrichment strategies (antibody, nanobody, and metal-based) for prion extraction and will combine enriched prions with recombinant prion proteins generated at the UMN protein core facility. Subsequent reactions will follow those used for prion amplification assays PMCA and RT-QuIC. Biosensor development will be performed in close coordination with the 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, both at the VDL and within our laboratories. 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 (includes personnel salary and fringe)

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. Generation of supporting data for patents filed relating to Activity 3 research.	1 June 2022

First Update March 1, 2020

As described in the overall project update, our major milestone with respect to Activity 3 was the outfitting of a new prion research laboratory and the successful implementation of RT-QuIC technology in the lab. This accomplishment was achieved through a key collaboration with the National Institutes of Health Rocky Mountain Laboratories. The RT-QuIC method can amplify CWD prions to a detectable level within hours of sample collection. In Jan 2020, we successfully detected CWD prions in a positive control sample within a 9-hour window. The RT-QuIC method can be used for live deer, hunter-harvested deer, and environmental samples. This method will serve as the basis for the development of our 3rd generation microfluidic CWD detection platform. Dr. Oh's laboratory has also successfully tested the SPR device described in Activity 3 (Outcome 1). During this work, we identified an affinity between prion proteins and specific particles. The significance of this finding is that we believe we can leverage the interaction between prion proteins and the particles in order to produce new tools for CWD detection.

Second Update September 1, 2020

As a result of research effort associated with Activity 3 (described above), we have made an important discovery that requires immediate attention. In short, we have identified particles that can effectively bind to the

misfolded CWD-causing prions. Our initial experiments lead us to believe that these particles can be leveraged for the development of rapid, low-cost, and low-tech CWD detection tools. Confirmatory lab tests are ongoing and, if promising, this work will result in an additional invention disclosure. Regarding the microfluidic flowcell development, we have constructed a functional prototype and initial experiments reveal that it is functioning as our engineering team anticipated. This prototype will allow for multiple experiments aimed at improving CWD diagnostic tool portability. A provisional patent will be filed on this device in the coming months and thus will require extensive testing over the extended project period.

Third Update March 18, 2021

In Oct 2020 we confirmed the binding potential of the particles referred to in our Sept 2020 update. This was a major step forward. Of significance is that the particle discovery was initially made by a graduate student supported by LCCMR funds and was the direct result of multi-disciplinary collaboration between engineers and biologists. Our research teams led by PI Larsen and Co-PI Oh worked diligently from Nov to Feb to perform a series of experiments confirming the particle / prion interaction. Results of experiments of the particle-based diagnostic test in Feb 2021 confirmed that we could deploy a CWD test in the field with potential results in less than 24 hrs. We worked with the MN DNR to coordinate the deployment of this first-of-its-kind CWD test. On March 8th 2021, we traveled to Rushford MN to perform field-based analyses of tissues secured by the annual DNR/USDA culling effort in SE MN. All research activities were reviewed and approved by UMN. Within 24 hours of arriving in Rushford, we confirmed that the test was functional. Day 2 of our expedition resulted in the identification of CWD positive tissue samples on site. These tissue samples were secured by the DNR/USDA during the culling effort and were independently validated by ELISA and IHC testing prior to our arrival at Rushford. A blinded analysis of tissues using our field-deployable test successfully identified the CWD positive animals. The test was successfully replicated with positive and negative control samples on days 3, 4 and 5. We believe that we are the first team to successfully identify CWD animals in the field and in less than 24 hours. This is a major advancement and we are working now to refine the method. Next steps will focus on the development of high-throughput applications that will allow for the screening of dozens of samples in parallel, with ramp-up capacity to hundreds of samples. Our team is planning to deploy the field-test to Tribal lands bordering Canada in early Fall of 2021 to further document feasibility and to perform CWD diagnostics in a more remote setting. We anticipate that the technology stemming from Activities 2 and 3 will lead to miniaturized diagnostic tools that can be used by non-experts.

Fourth Update September 1, 2021

Two provisional patents have been filed on the MN-QuIC test. We have completed internal validation of the field-deployable MN-QuIC test using lymph node tissues. The test is 100% accurate with RT-QuIC, ELISA, and/or IHC tests of the same tissues. This result indicates that the MN-QuIC test has great promise for field-based testing of deer. We envision the test would be best used at the county level and could be used to screen harvested deer with a 24 hour turnaround time. Additional testing of MN-QuIC for tissues / samples that can be taken from live deer are underway. The MN-QuIC test leverages gold nanoparticles, a reagent that is becoming increasingly common for a variety of diagnostic tests. Our research shows that amplification of CWD positive material results in a red color when mixed with gold nanoparticles and a blue or purple color when mixed with CWD negative samples. During the summer of 2021 we made an important discovery concerning the microfluidic platform that we are engineering for CWD testing. Our research indicates that we can amplify and detect CWD positive tissues in less than four hours of microfluidic-based testing. This avenue of research was part of the original testimonies that Dr. Larsen provided in 2018 and 2019. The research is producing very promising results now and it is possible the device that we have engineered would allow for on-site testing with same-day results. The prototype is approximately the size of a domino. We are working to file a provisional patent on the technology now with OTC.

Fifth Update March 1, 2022

We have completed the internal validation of the 24-hour MN-QuIC test and results show that it has a diagnostic sensitivity of 95.7% and specificity of 100%. This result is exciting because it shows the MN-QuIC test is as good

as, or better than, the current ELISA and/or IHC tests for CWD. We have incorporated these results into our manuscript and will now submit for publication to Scientific Reports. Regarding our microfluidic testing device, we have fine-tuned the platform such that multiple prototypes can be easily produced. Additional tests continue to show that it can identify CWD positive tissues in less than 4 hours of runtime and we are not seeing evidence of false-positive results when using CWD negative tissues. We believe that the microfluidic device will provide a pathway to a deer-side test that can be used by state or federal agencies to better control the disease.

Update as of June 30, 2022:
Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:
Activity 3 has produced incredible results pertaining to rapid field-deployable CWD diagnostics. Our team is now on a path to a deer-side test that could be completed within less than 4 hours. It is possible, based on our latest data, that the test could be completed in 2 hours. This research is focused on the prototype discussed at the end of our previous update. We have now named the test Micro-QuIC. We are finalizing the patent submission of Micro-QuIC, to include our most recent data. We have also identified unique properties of CWD positive samples that can be leveraged in our Micro-QuIC platform in such a way as to produce multiple layers of confirmatory testing on the same device. What this means is that an initial CWD positive result could be confirmed with additional mechanisms on the same device. We believe this approach will result in an incredibly powerful portable assay that could be used for on-site CWD diagnostics, either by state and tribal agencies or by hunters themselves. We are excited for this potential and continue to refine the design of Micro-QuIC. Our MN-QuIC assay, described above, is being submitted for official USDA validation. Our team has performed hundreds of CWD tests using MN-QuIC and the assay is performing exceptionally well. The test is described in a recent peer-reviewed publication entitled “A field-deployable diagnostic assay for the visual detection of misfolded prions”. Both Micro-QuIC and MN-QuIC represent monumental steps forward for CWD diagnostics. We are now working with the UMN OTC to explore commercialization options for these assays.

Final Report Activity:
The research efforts devoted to Activity 3 ultimately led to the achievement of the overall goal of the project, to develop new diagnostic tools that will aid in the fight against CWD. We invented a 24-hour portable CWD test, called MN-QuIC, that is currently undergoing USDA validation. Developing a new diagnostic assay that has reached the milestone of entering the USDA validation process is a major step forward. Additionally, we invented a microfluidic diagnostic platform the size of a domino that is capable of testing for CWD in less than 4 hours. We believe that we can combine these two inventions into a single portable diagnostic tool that state, tribal, and federal agencies can use for the rapid field-based detection of CWD prions in both biological (e.g., deer tissues and fluids) and environmental (e.g., feces, swabs of feeders, water tanks) samples. We have filed multiple provisional patents for these inventions and are working to commercialize them to make them available to stakeholders. We are optimistic that the research output from Activity 3 will provide real-time surveillance capacity for CWD, thus cutting down on testing bottlenecks and allowing stakeholders to fight the disease in new ways. In addition to the new tests invented by our team, Activity 3 led to RT-QuIC capacity in Minnesota. RT-QuIC is an ultra-sensitive prion detection assay that is capable of detecting CWD prions in biological and environmental samples. We expanded RT-QuIC testing capacity within our research laboratory and have used the test to assist the DNR and BAH with CWD testing needs (e.g., the Beltrami County deer carcass disposal site). We are now working to provide the public with access to the RT-QuIC test for deer muscle samples. We hope this will help stabilize / increase deer hunting participation within the state, an important population management tool for fighting CWD.

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

Regarding dissemination of our CWD diagnostic development efforts facilitated by LCCMR funding, Dr. Larsen has presented at multiple local, state, and federal venues. Including:

State and Federal Testimonies

- Interior Department Appropriations. Congresswoman Betty McCollum Listening Session. FY 2021. On behalf of the University of Minnesota. Bell Museum of Natural History. 24 Feb 2020
- MN State Legislature Testimony. Senate Environment Finance Committee. 18 Feb 2020
- MN State Legislature Testimony. House Environment Finance Committee. 18 Feb 2020
- United States 116th Congress Testimony. House Appropriations Subcommittee on Interior, Environment, and Related Agencies. 17 Oct 2019 www.congress.gov/event/116th-congress/house-event/110072
- MN Congressional Joint Meeting Testimony. House and Senate Environment and Natural Resources Finance Division. 29 Oct 2019

Invited Presentations

- Chronic Wasting Disease: an immediate threat to cervid heritage. University of Oklahoma. Sam Noble Museum. 20 May 2020 (remote lecture)
- The Biology of Chronic Wasting Disease. Minnesota Elk Breeders Association Annual Meeting. Jan 2020.
- Chronic Wasting Disease. Department of Biology, Winona State University. Winona MN, Oct 2019.
- A One-health collaboration: cutting-edge genomics in the jungles of Borneo. Faculty of Science and Technology. University of Malaysia, Sarawak. Malaysia. Aug 2019.
- Chronic Wasting Disease: diagnostic development. Minnesota Department Natural Resources, Annual Midwest Wildlife Committee Meeting. Duluth MN, May 2019.

The CWD diagnostic development team has been very active with extension and outreach activities where we highlight our LCCMR research efforts and acknowledge LCCMR. Note, these activities were funded by startup funds awarded to Dr. Larsen, RAPID Ag response funds, or funds secured from the University of Minnesota specifically for outreach and education. LCCMR funds are being used exclusively for the research efforts described above however we acknowledge LCCMR during these events:

Extension and Outreach

- Dr. Larsen presentations organized by UMN Government Relations. Minnesota Rotary Club Presentation Series (Organized by UMN contract lobbyists Weber and Johnson). Nov 2019 to present. The Biology of Chronic Wasting Disease. Provided oral presentations to Red Wing, Faribault, Rochester, Chanhassen, and Minneapolis Rotary Clubs. ~200 MN civic leaders attended as of 29 May 2020.
- Minnesota Science Museum. “Behind the Scenes” events. The science of Chronic Wasting Disease. Augmented reality displays and handouts. 1,777 attendees from the general public. Jan and Feb 2020.

- Minnesota Clean Water Council Meeting. Oral presentation on the environmental hazards associated with Chronic Wasting Disease prions. 12 council members. Jan 2020.
- Amish Community Outreach. Biology of Chronic Wasting Disease. Harmony, MN. ~20 Amish hunters. Dec 2019.
- Southeast Asian Community Outreach. Biology of Chronic Wasting Disease. St. Paul, MN. ~25 Hmong hunters, 4 state legislators, 1 St. Paul city councilman. Dec 2019.
- Minnesota Legislative Mini-Session, Winona, MN. Chronic Wasting Disease augmented reality displays and educational handouts at Winona Statue University. ~200 attendees: general public, MN legislators, WSU faculty, staff. Oct 2019.
- Bell Museum of Natural History. Conference on Chronic Wasting Disease. St. Paul, MN. ~200 attendees, general public, UMN administration and faculty, MN Dept. of Natural Resources, Board of Animal Health, Elk Breeders Association. Sept 2019.
- Eagle Bluff Environmental Learning Center, "Chronic Wasting Disease of Deer", Lanesboro, MN. 70 attendees, general public. Sept 2019.

Our work has also received the following media attention and we have made every attempt to ensure reporters acknowledge LCCMR:

- Star Tribune. University of Minnesota achieves milestone for CWD testing of wild and tame deer. 22 Feb 2020.
- UMN News. Going mobile to stop pathogens. 5 Feb 2020.
- UMN News. Research team achieves CWD testing milestone. 18 Feb 2020.
- Outdoor Life. Cutting-edge test is able to detect Chronic Wasting Disease in Live deer. 21 Feb 2020.
- UMN Profiles. Into the Forest: A leading-edge mobile lab opens the door for discovery, education, and collaboration. Fall 2019.
- CBS. WCCO Minnesota. Television Interview with Bill Hudson. Oct 2019. Chronic Wasting Disease in Minnesota.
- Star Tribune. Wasting disease puts deer farms under fire. 26 Nov 2019.
- ABC. KSTP Minnesota. Local airing of my federal Congressional testimony. "We are at a critical moment in the fight against CWD". 17 Oct 2019.

Publication acknowledging LCCMR funds

M. Vala, C. T. Ertsgaard, N. J. Wittenberg, S-H. Oh. 2019. Plasmonic sensing on symmetric nanohole arrays supporting high-Q hybrid modes and reflection geometry. American Chemical Society Sensors, 4,12, 3265-3274.

Second Update September 1, 2021

Dissemination activities relating to public outreach have been impacted by the ongoing pandemic. Nevertheless, we have adapted our activities to include virtual presentations. We have completed a webinar series focused on the biology of CWD and this series includes lectures by PI Larsen, Mr. Cory Anderson (UMN CIDRAP), and Erik Hildebrand and Christopher Jennelle (MN DNR). All presentations are available on our MNPRO outreach website (https://mnpro.umn.edu/outreach). PI Larsen provided a project update to the legislature during testimony at the joint House and Senate Environment and Natural Resources Finance hearing held on 1 September 2020. Dr. Larsen acknowledged LCCMR during the webinar series and his testimony. Additional publications stemming from our work or currently in various stages of development and we anticipate submission of these beginning in December 2020 and January 2021.

Third Update March 18, 2021

In October 2021, the prion-focused research team developed and distributed several hundred booklets that describe the biology of CWD and how the disease is transmitted in deer. These booklets were distributed throughout SE Minnesota by Dr. Larsen prior to hunting season, with drop-off locations consisting of venues where deer hunting licenses are sold and where our Amish hunting communities purchase their licenses and included Winona, Houston, Spring Grove, Mabel, Harmony, Preston, Lanesboro, Rushford, Lewiston, and St.

Charles. SE Minnesota was targeted due to the prevalence of CWD in wild deer populations in that part of the state. Dr. Larsen performed a virtual outreach activity during the field trip to Rushford on March 10th 2021. He provided a presentation on CWD to the Rochester Lifetime Learners club via zoom. This event was significant because it was performed in the same room where our field-test was setup and he was able to talk with the public about why such a field test was important for CWD surveillance. Dr. Larsen, Dr. Tiffany Wolf, and Marc Schwabenlander also traveled to the Harmony and Canton areas in SE MN on March 9th and March 12th to meet with Amish community leaders about CWD and to schedule a CWD outreach event to be held near Canton in early April. All outreach activities have and will continue to follow state and federal guidelines for limiting the transmission of COVID-19. The prion-research group has submitted one manuscript for peer-review (currently in review, preprint provided here https://www.biorxiv.org/content/10.1101/2021.03.03.433751v1). A second manuscript focused on the detection of CWD in deer muscle tissues will occur the week of March 22nd, and a third manuscript is planned for submission in May 2021 that describes the particle-based diagnostic technique (as approved by UMN OTC as we navigate patent submission).

Fourth Update September 1, 2021

We have made significant progress with respect to scientific publications stemming from our research and outreach efforts. Several manuscripts have undergone peer-review and have been published, available here:

RT-QuIC based detection of CWD in deer muscles:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8373970/

Comparison of CWD testing methods, including RT-QuIC:

https://meridian.allenpress.com/jwd/article/doi/10.7589/JWD-D-21-00033/472442

The MN-QuIC manuscript is currently being peer-reviewed yet has been made available to the public (with approval by the UMN Office of Technology and Commercialization) here:

MN-QuIC assay: https://www.biorxiv.org/content/10.1101/2021.11.22.469560v1

In addition to publications, outreach activities have consisted of seminars and lectures made by Drs. Larsen and Wolf and Marc Schwabenlander to students at the University of Minnesota, state-level agencies including the DNR and BAH, and county commissioners (St. Louis Co. [7 September] and Winona Co. [28 September]). Dr. Larsen also provided testimony during the House Environment and Natural Resources committee meeting on 14 September. Our team also assisted with outreach events in Beltrami Co. on the 28th and 29th of September, organized by the DNR and BAH.

Drs. Larsen and Wolf held a special CWD panel discussion at the annual American Veterinary Medical Association meeting. The panel included experts from tribal, state agency, and academic institutions and was focused on the human dimensions of CWD.

Additional updates pertaining to our research and outreach activities are made available on our website: mnpro.umn.edu

Fifth Update March 1, 2022

Dr. Larsen continues to provide project updates to legislators and the public via state testimonies, having provided two testimonies since our last update:

- MN State Legislature Testimony. House Environment and Natural Resources Finance Division. 14 Feb 2022.
- MN State Legislature Testimony. House Environment and Natural Resources Finance Division. 5 Sept 2021.

Our research efforts were highlighted in multiple media releases between Sept 1st 2021 and March 1st 2022:

- The Bemidji Pioneer. Hines deer farmer fights Board of Animal Health after illegal dumping of CWD-infected deer carcasses. 9 Feb 2022.
- MPR. (story by Dan Gunderson and live on air interview with Cathy Wurzer). New Chronic Wasting Disease test: game-changer or unproven? 4 and 5 Nov 2021.
- Minnesota Daily. University Veterinary Science faculty lead CWD research. 3 Nov 2021.
- MPR. Hunters want more urgency in state's CWD response. 29 Oct 2021.
- NBC: KARE11 (television interview). Managing CWD in farmed and wild deer populations. 22 Sept 21.
- NBC: KBJR6. Chronic Wasting Disease and deer hunting in Minnesota. 8 Sept 2022.
- Duluth News Tribune. At war with CWD, St. Louis County aims for moratorium on deer farms. 8 Sept 2021.

Our team provided CWD information to both the St. Lous County and Winona County board of Commissioners. We also presented at two public town hall events organized by the DNR and BAH in Beltrami Co. Because CWD is a national disease, effective strategies of fighting the disease requires a coordinated effort across states. Although not supported by LCCMR funds, the MNPRO team traveled to Texas for outreach events to the public and to Texas state agencies tasked with managing CWD:

- Public presentation to Texas ranchers managing deer herds throughout west Texas. Presentation entitled The Biology of Chronic Wasting Disease. Barn Door Restaurant, San Antonio, TX. 1 March 2022.
- Texas Deer Association and Texas Deer Breeders. Presentation on the ecology of CWD prions and diagnostic tools for CWD. Double C Ranch and Smitty's Restaurant, Lockhart, Texas. Oct 2021
- Ceaser Kleberg Wildlife Center. Presentation on the biology of CWD and MNPRO research advancements. Duval Ranch, Texas. Oct 2021.
- Winona County Board of Commissioners. Presentation of the biology of CWD, fielded questions from the Board. 28 Sept 2021
- Joint DNR, BAH, and MNPRO outreach events in Beltrami Co. Public outreach on CWD in light of carcass dump site on public lands. Bemidji High School and High School. 28 and 29 Sept 2021.
- St. Louis County Board of Commissioners. Presentation of the biology of CWD, fielded questions from the Board. 7 Sept 2021.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Our in person outreach efforts have been impacted by the COVID pandemic, nevertheless, to date we estimate that we have connected with over 21,000 members of the public regarding the science of CWD (including 7,000 through in-person and virtual outreach events and 14,000 individuals through online content). We are working closely with the tribal nations, Minnesota DNR, Minnesota BAH, and several state legislators regarding a number of CWD-related issues in Minnesota.

<u>Media Coverage</u>

- MPR. (story by Dan Gunderson). State funding for MNPRO and CWD research outcomes. 26 May 2022.

State Testimonies

- MN State Legislature Testimony. Joint Meeting Testimony. House and Senate Environment and Natural Resources Finance Division. 10 May 2022.
- MN State Legislature Testimony. Joint Meeting Testimony. House and Senate Environment and Natural Resources Finance Division. 15 March 2022.
- MN State Legislature Testimony. House Environment and Natural Resources Finance Division. 14 Feb 2022.

Extension and Outreach

- Iskigamizige-Giizis Maple Sugar Moon Pow Wow. CWD biology. 25 tribal hunters. Apr 2022.
- UMN College of Veterinary Medicine Issues Class. CWD diagnostic development. 25 CVM students. Mar 2022.

Final Report Activity:
Collectively, we presented at or held 43 outreach events and estimated that we connected with over 28,000 members of the public regarding the science of CWD (including ~7,500 through in-person and virtual outreach events and ~21,000 individuals through online content). In addition to these events, our work was frequently highlighted in the media with over 50 news articles and television and radio interviews. We developed and disseminated an array of outreach materials including: fact-sheets translated into multiple languages, websites with helpful CWD information, animations and virtual posters to help the public better understand the biology of CWD, and a webinar on the science of CWD. We have also produced five publications in peer-reviewed journals and 24 presentations at scientific conferences or venues, collectively advancing the science of CWD surrounding diagnostics and surveillance.

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 be used within our prion research cluster throughout the lifetime of each unit.

**As noted in our revised budget amendment, several of the capital expenditures originally listed here are not needed as they are available within UMN (i.e., Agilent bioanalyzer).

December 7th Update
As described in our latest budget amendment, we are listing an Eppendorf EP-Motion 5073 machine that will allow us to dramatically increase the throughput of our RT-QuIC and MN-QuIC testing. This machine is a liquid handler that will pipette samples into a 384 well plate. MNPRO now has 6 RT-QuIC machines that we are currently using for 96 well plates. Increasing to 384 well plate capacity means that MNPRO will have the

capacity to analyze at least 564 deer every 24-48 hours. The increase in testing throughput is needed to screen not only tissues but also environmental samples (i.e., feces, soil and water) that consist of hundreds to thousands of samples.

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 assist with securing deer samples throughout Minnesota for diagnostic development. They will also help to validate any diagnostic test emerging from the research discussed herein.

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 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 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 sensitive and accurate 3rd generation CWD diagnostic tools. These tools will be validated at the UMN Veterinary Diagnostic Lab 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 generation CWD diagnostic tools will be of great importance as they will help to provide a real-time assessment 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, 2023

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-Final
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: 3 Years 29 December 2022
For the period ending: June 30, 2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Revised Budget	Amount Spent	Balance
BUDGET ITEM				
Personnel (Wages and Benefits)	$ 930,768	$ 1,083,096	$ 1,083,096	$ -
Professional/Technical/Service Contracts				
Dr. Helen Dooley, prion-specific nanobody development, $80,000	$ 80,000	$ 80,000	$ 80,000	$ -
Equipment/Tools/Supplies				
Supplies for RNA extraction, protein purification, RT-QuIC reagents,	~~$ 47,208~~	$ 64,164	$ 64,164	$ -
Microfluidic flowcell engineering, SPR reagents, biosensor	~~$ 34,000~~	$ 37,492	$ 37,492	$ -
RNA-seq library prep fees, mRNA and microRNA assays, Illumina	~~$ 137,000~~	$ 29,473	$ 29,473	$ -
-20 freezer, $1,400	~~$ 1,400~~	$ 1,391	$ 1,391	$ -
Glass door refrigerator for prion lab (VWR) $4,780	$ -			$ -
Benchtop centrifuge, $4,036	$ 7,410	$ 7,410	$ 7,410	$ -
Prion Lab equipment, glass-ware, consumables, general western	~~$ 119,154~~	$ 80,794	$ 80,794	$ -
Quibit Fluorometer and reagents for RNA quantification, $4,000	~~$ 4,000~~	$ 2,997	$ 2,997	$ -
Materials and supplies for protein amplification assays, antibody-	$ -			$ -
Recombinant protein production, $70,000	~~$ 70,000~~	$ 42,951	$ 42,951	$ -
Synthetic antibody production, $60,000	~~$ 50,000~~	$ 5,757	$ 5,757	$ -
Protein analyses at UMN Proteomics Core, $20,000	~~$ 20,000~~	$ 8,083	$ 8,083	$ -
Tissue collection and preservation, $10,000	~~$ 10,000~~	$ 491	$ 491	$ -
Capital Expenditures Over $5,000				
-80 freezer for RNA sample storage, $15,000	$ 21,975	$ 21,975	$ 21,975	$ -
-80 freezer for cervid tissue storage, $15,000	$ 19,550	$ 19,550	$ 19,550	$ -
BMG Labtech Omega Microplate Reader (RT-QuIC analyses;	$ 29,100	$ 29,100	$ 29,100	$ -
Allegra x-30R Beckman Coulter Refrigerated Centrifuge, $20,000	$ 9,870	$ 9,870	$ 9,870	$ -
BioRad ChemiDoc MP Imaging System (western blot analyses and	$ 34,565	$ 34,565	$ 34,565	$ -
CWD Diagnostic Equipment for Veterinary Diagnostic Lab	$ 175,000	$ 175,000	$ 175,000	$ -
Eppendorf epMotion 5073	~~$ -~~	$ 69,841	$ 69,841	$ -
Travel expenses in Minnesota				
Travel to attend in-state conferences to disseminate results,	~~$ 3,000~~	$ -		$ -
Other				
	$ -		$ -	$ -
COLUMN TOTAL	**$ 1,804,000**	**$ 1,804,000**	**$ 1,804,000**	**$ -**

UNIVERSITY OF MINNESOTA
Driven to Discover℠

Minnesota Center for Prion Research and Outreach

"Development of Advanced Diagnostic Tests for Chronic Wasting Disease"

Project Goal

To launch research projects aimed at developing new CWD diagnostic tools for rapid and sensitive animal testing and environmental monitoring.

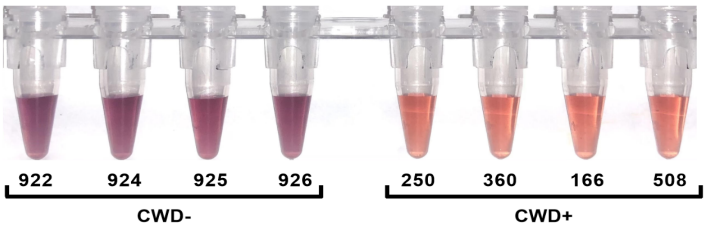
Project Implementation Strategy

Make rapid CWD test discoveries through multidisciplinary research
Bring together diverse expertise in animal health, cellular and protein biology, nanotechnology engineering, and human and animal protein-misfolding diseases with a common goal of advancing CWD diagnostic testing.

Establish a think-tank environment
Create an incubator for cutting edge science and invent new technologies for CWD prion diagnostics.

Coordination and collaboration
Work with state and tribal agencies and engage stakeholders through research and outreach initiatives.

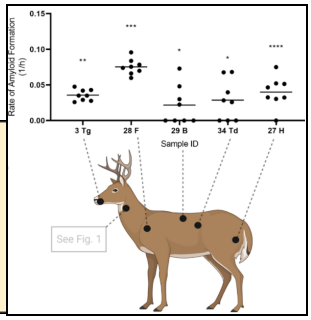
Major Discovery in 2020



Our team invented a new diagnostic test for CWD. This new assay, "MN-QuIC", is portable and generates a color change of red for a positive CWD result and blue for negative in about 24 hours. We have published the test and are now entering USDA validation.

Additional lines of ongoing R&D include:

- A 4-hour, microfluidic CWD test
- A deer-side CWD test
- Four associated provisional patents.



MNPRO established RT-QuIC testing in Minnesota and developed the world's first protocol to test deer muscle deer muscle for CWD.

Chronic Wasting Disease

Chronic wasting disease (CWD) is an emerging prion disease of deer. CWD is spreading through deer populations in Minnesota and throughout North America and there is growing concern of transmission to wildlife and humans.

The Wagenius Effect

To Rep. Jean Wagenius, the need was not just for advancing CWD diagnostics. Wagenius wanted the UMN to *immediately* start educating the public about CWD.

MNPRO led in-person and virtual community education events reaching over 28,000 people since 2019.

MNPRO invented multiple educational tools, including an anatomically accurate 3D deer head model for CWD sample collection training.

Funding for this project was provided by the Minnesota Environment and Natural Resource Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).