NDSU NORTH DAKOTA STATE UNIVERSITY

NOTICE OF IBC APPROVAL

8/14/2019

Dharmakeerthi Nawarathna Electrical & Computer Engineering

B20006-Efficacy of micro RNA testing for identification of personalized weight management strategy in obese and overweight individuals

Research location(s): Ehly 202

The NDSU Institutional Biosafety Committee has reviewed and approved the above referenced protocol under the category:

Exempt rDNA NIH Section III-F-1

Approval Date: 8/13/2019
Annual Update Due: 7/1/2020
Expiration Date: 8/12/2024

As project director, you are responsible for conducting the protocol as described, utilizing BSL-2 containment. The IBC retains the right to conduct inspections of the research facilities at any time.

Additional reporting to the NDSU IBC is required for this project:
• when making changes in personnel and/or any approved procedures, locations or agent(s). Submit the IBC Request for Change Form prior to implementation of the change(s). Please note that changes may require re-evaluation of the risk assessment and biosafety level for appropriate containment.
• in the event of significant problems concerning exposure or containment of the agent(s) involved. Submit the IBC Adverse Event Reporting Form within 24 hours of awareness of the event.
• if the study will continue beyond the approval period. Submit the Progress Report Form for IBC review approximately one month prior to the expiration date. In addition, annual status updates are required (submit the IBC Annual Update/Completion Report Form). You should receive a reminder approximately one month prior to each report date.

NOTE Report all exposure incidents (e.g., via skin puncture, mucous membrane, non-intact skin, or other means) immediately to the Safety Office at 701-231-7759 for evaluation and follow up. Also notify the IBC of the incident within 24 hours.

INSTITUTIONAL BIOSAFETY COMMITTEE
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8908 | Fax 701.231.8098 | ndsu.research@ndsu.edu

Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

NDSU NORTH DAKOTA STATE UNIVERSITY

Institutional Biosafety Committee
Research Integrity and Compliance Office
Research 1-1735 Research Park Drive
www.ndsu.edu/ibc

Institutional Biosafety Application
Questions about this form? Call 231-8908
Submit form to ndsu.ibc@ndsu.edu

IBC USE ONLY

Protocol # B20006 Review ◉ Designated ○ Full Board Approval Date

Protocol Title: Efficacy of micro RNA testing for identification of personalized weight management strategy in obese and overweight individuals

Principal Investigator Name: Dharmakeerthi Nawarathna

Department: Electrical and Computer Engineering

Email: dharmakeerthi.nawara@ndsu.edu

Phone: 701-231-7916

Funding Agency: Sanford-NDSU seed award

Anticipated Start and End Date: 08/15/2019- 08/14/2024

Other Approvals

IACUC Approval: No Protocol Number:

IRB Approval: Pending Protocol Number:

Radioisotopes- No Contact the Radiation Safety Committee

Biological Toxins, Nanomaterials, or Chemicals: No Contact the Laboratory and Chemical Safety Committee

USDA-APHIS or BRS Permit: No Please attach a copy of the applicable permit.

Research Team and Required Training

All research team members should be listed below. Training requirements are based on the agents/materials used in the protocol. Required training courses (www.citiprogram.org)

All protocols-**Basic Biosafety Training Course**
Protocols involving Recombinant or Synthetic Nucleic Acids-**NIH Recombinant DNA Guidelines Course**
Protocols involving Human blood, bodily fluids, tissues, or cells-**OSHA Bloodborne Pathogens Course**

IBC Office staff will enter training expiration dates

Add Name	Name	Role in Protocol	Basic Biosafety	NIH Recombinant DNA Guidelines	OSHA Bloodborne Pathogens
Remove Name	Dharmakeerthi Nawarathna	PI	7/16/2022	7/16/2022	7/16/2022
Remove Name	Vidura Jayasooriya	Graduate Student Researcher	3/27/2022	3/27/2022	3/27/2022
Remove Name	Beth Ringwelski	Graduate Student Researcher	4/1/2022	4/1/2022	7/15/2022
Remove Name	Rounak Pokharel	Graduate Student Researcher	8/8/2022	8/8/2022	8/8/2022

Project Description

In terms that would be understandable to the general public, provide a brief overview of the goals and objectives of the project; citing references as applicable

Project Goals -*Briefly describe your research project goal(s).*

PI, Nawarathna has recently developed a sensor that could detect (ex vivo) nucleic acid biomarker (circualting microRNA and antigen) molecules in blood and serum samples. The goal of this study is to demonstrate sensor's ability to accurately detect the presence and number of copies about 3-4 microRNA (miRNA) targets. We will use commercially available serum samples and commercially synthesized miRNA/DNA molecules. Molecules will be spiked to the serum and developed sensor will be used to detect the presence of each miRNA target as well as there quantity.

Procedures*-Briefly describe your laboratory and field procedures involving biohazardous agents.*

Commercially available serum samples will be purchased and used in experiments. Researchers will use 100% serum or 10%, 1%, 0.1% 0.01% or 0.001% serum by volume (diluted with commercially available DI water, phosphate buffered saline (PBS) or TrisEDTA (TE)) in experiments. MiRNA molecules will be commercially synthesized (by IDT DNA or similar company) and used in experiments. In experiments, 0.1-5mL of serum or diluted serum samples will be used, a known miRNA molarity (1mM- 1aM) will be spiked and mixed by vortexing. We will then pipette an equal molarity of commercially synthesized complementary (to the miRNA target) DNA molecules (by IDT DNA or similar company) and hybridize miRNA and DNA molecules in a lab incubator (at 55C for 15 min). The sample will then be pipetted directly on the sensor surface, apply small electric potential (0-10 V, 0-20 MHz) and measure fluorescence (using fluorescence microscope) or electrical impedance (commecially available impedance analyzer). Finally, molarity of miRNA will be calculated using the magnitudes of fluorescence intensity or electrical impedance.

References-*List any literature references relevant to this application.*

(1) Lautner, G. and Gyurcsányi, R.E., 2014. Electrochemical detection of miRNAs. Electroanalysis, 26(6), pp.1224-1235.
(2) Yin, B.C., Liu, Y.Q. and Ye, B.C., 2012. One-step, multiplexed fluorescence detection of microRNAs based on duplex-specific nuclease signal amplification. Journal of the American Chemical Society, 134(11), pp.5064-5067.

Study Type

Please check the appropriate box(es) that apply to the project described above and provide additional information as requested.

☒ **Recombinant or Synthetic Nucleic Acids**

Check the NIH Guidelines section that is applicable to this project.

☒ **Section III-F Exempt Experiments**

☒ III-F-1*Experiments involving nucleic acids that cannot replicate in any living cell, cannot integrate into DNA, and do not produce a toxin with an LD50 of less than 100ng/kg body weight*

☐ III-F-2 *Experiments not conducted in organisms, cells, or viruses and that have not been modified or manipulated to render them capable of penetrating cell membranes*

☐ III-F-3: *Recombinant or synthetic nucleic acids that exist solely of the exact nucleic acid sequence from a single source that exists in nature*

☐ III-F-4, III-F-5 *Nucleic acids from a prokaryotic or eukaryotic host, when propagated only in that host (or closely related strain of the same species*

☐ III-F-6 *DNA segments from different species that exchange DNA by known physiological processes (must consult Appendix A of the Guidelines for lists of designated exchangers.*

☐ III-F-7 *Genomic DNA molecules that have acquired a transposable element, if the transposable element does not contain any recombinant or synthetic DNA*

☐ III-F-8 *Experiments that do not present a significant risk to health or the environment (You must consult Appendix C of the Guidelines for specific categories and exemptions. Examples include Escherichia coli K-12 host-vector systems, Saccharomyces host-vector systems, purchase or breeding of transgenic rodents.*

☐ **Section III-E IBC Notice Simultaneous with Initiation**

☐ **Section III-D IBC Approval Before Initiation**

☐ **Section III-C IBC, IRB and RAC Approval Before Initiation**

☐ **Section III-B NIH/OBA and IBC Approval Before Initiation**

☐ **Section III-A RAC Review and NIH Director and IBC Approval Before Initiation**

Sub-Sections-*Please indicate applicable sub-section(s) of the NIH Guidelines*

Add Row	Source of DNA	Nature of inserted DNA Sequence(s)	Vector(s) Indicate antibiotic trait(s)	Host(s)
Delete Row	Commercially synthesized	DNA will not be inserted to any cells	No viral vectors will be used	N/A

Please check the applicable boxes

☐ In vivo use

☐ Expression of a foreign gene

☐ Genes coding for the biosynthesis of molecules toxic for vertebrates

☐ Growth of large-scale (>10L) cultures containing Recombinant or Synthetic Nucleic Acids

Procedures-*Describe specific procedures involving the collection, creation, use, analysis and transport of recombinant and synthetic nucleic acids.*

Nucleic acid samples (miRNA and DNA) will be purchased and vendor is responsible for safe transporting to the PI's lab. In the PI's lab, samples will be stored in the appropriate temperature to minimize the degradation. Nucleic acid samples used in miRNA detection experiments will be carefully handled by the trained researchers during and after experiments outlined above. All the experiments will be performed in the BSL-2 section of the PI's lab at Ehly 202. The waster materials will safely be disposed according to the NDSU biohazard disposal guidelines.

Risks-*Describe the risks for human, animal or plant/environmental health posed by these materials and what safety measures will be taken.*

Since the nucleic acid molecules especially miRNA/DNA could degrade quickly, it will not pose any safety concern to human, animal, plant or environment.

☐ **Infectious Agents**

☐ **Dual Use Research of Concern (DURC) (e.g. botulinum toxin)**

☒ **Human Blood, Bodily Fluids, Tissues or Cell Cultures**

Add Line	**Description** (e.g. whole blood, spinal fluid, tissue, cell lines)	**Source** (e.g. hospital, university, federal repository , commercial vendor or colleague)	Product Number or Approximate Date of Acquisition
Remove Line	serum samples	commercial vendor	product number: ICSERS2ML or similar from Innovative research

Collection and Transport. *Describe specific procedures involving the collection and transport of human blood, bodily fluids, tissues and human cell lines.*

Commercially viable human serum samples of healthy individuals will be purchased.

Use and Analysis. *Describe specific procedures involving the use and analysis of human blood, bodily fluids, or cell lines.*

Serum samples will be purchased and vendor is responsible for safe transporting to the PI's lab. In the PI's lab, the sample will be stired in the appropriate to minimize the degradation. Serum samples used in miRNA detection experiments will be carefully handled by the trained researchers during and after experiments outlined above. All the experiments will be performed in the BSL-2 section of the PI's lab at Ehly 202. The waster materials will safely be disposed according to the NDSU haphazards disposal guidelines.

Risks. *Describe risks for human, animal and environmental health posed by these materials, and what measures will be taken to ensure safety.*

Human serum from Innovative Research is collected from healthy donors. The plasma (collected via apheresis) is processed into serum at FDA-licensed facilities located in the United States. Serum is filtered and cell culture tested for mycoplasma, endotoxin, and sterility.

Viral Testing
Each unit is tested for the standard FDA-required viral markers, and found negative for HBsAg, HCV, HIV-1, HIV-2, HIV-1Ag or HIV-1 NAT, ALT, and syphilis using FDA- approved methods. For these reasons, if proper handling and disposal procedures were followed, there will be a minimum risk to human, animal and environments.

Hepatitis B Vaccine Series. *The Hepatitis B vaccine series is available and must be offered to all personnel who have routine exposure to human blood, or other potentially infectious materials. Please refer to the* **NDSU Exposure Control Plan** *or contact the* **Safety Office** *for more information.*

Please list the names of individuals that will have routine exposure to human blood, bodily fluids or tissues while working on this project.

Add Name	Name	Hepatitis B Vaccine Status?
Remove Name	Vidura Jayasooriya	Vaccinated
Remove Name	Beth Ringwelski	Vaccinated
Remove Name	Rounak Pokharel	Vaccinated

Project Site(s)

Please list all sites and locations to be used (e.g. locations used for culture, creation, analysis, manipulation and storage of biohazardous agents. Include Core Facilities and locations for animals and plants used in conjunction with this project).

Add Location	Building/Location	Room #	Activity/Use	Biosafety Level
Remove Location	Ehly Hall	202	Research Lab	BSL-2

Projects that have a containment level of BSL-2 or higher are required to have a laboratory specific Biosafety Manual. In addition all locations where BSL-2 or higher work is being conducted are required to have the space inspected by the NDSU Biological Safety Officer.

Does this project have a containment level of BSL-2 or higher? (•) Yes ( ) No

Biosafety Laboratory Inspection: Yes

Laboratory Specific Biosafety Manual: Yes

Laboratory Inventory of Infectious Agents or Toxins: Yes-on file in the lab

Containment

Describe containment measures to prevent occupational or public health exposure to and/or release of biohazardous agents to the environment

☒ **Aerosols-**Procedures that may generate aerosols will be used in this project.

Please identify all experimental procedures that may generate aerosols and describe how they will be minimized or contained. Examples of procedures include: grinding, sonicating, blending, shaking, vortexing, vigorous pipetting, centrifuging, intranasal inoculation of animals, and opening pressurized containers.

Care will be taken, especially ejecting disposable pipette tips and avoid aerosol formation. The centrifuge tubes will not be over filled and researchers will wipe the outside of tips with disinfectants after they are filled and sealed. Researchers will never use mouth pipetting in this project. Vortexing and shaking will be done in the biological safety cabinet to minimize the aerosol generation.

☒ **Biological Safety Cabinet-**A BSC or another type of containment chamber will be used in this project.

Please indicate the location and type of containment cabinet/chamber being used.

Biological Safety Cabinet is located in the far end corner of the BSL-2 room. Note the certification of BSC has been expired and we made a

Date of last certification: Aug 2, 2018

☐ **Plants**-This project involves the use of plants.

Waste

Please check the types of waste that will be generated

- ☐ Sharps
- ☒ Liquids
- ☒ Solids
- ☐ Animal Carcass
- ☐ Plants
- ☐ Other

If other, please explain:

Disinfection/Decontamination-*Describe procedures for disinfection/decontamination of the various types of wastes that will be generated.*

Household bleach (1:2 by volume= waste: bleach) will be added to the waste materials after experiments and kept in the room temperature for about 15 min and dispose according the NDSU was disposal procedure. After each experiment, work surfaces (e.g., table tops, pipettes etc.) will be spayed with 75% ethanol (by volume) and wait about 5 min and clean with paper-towels. After each experiment, sensor surface will be sprayed with 100 % bleach, wait about 15 min, washed with DI water and dry in room temperature.

Disposal-*Describe procedures for disposal of biohazardous wastes.*

PI will work with NDSU biological safety office to properly dispose the hazardous materials.

Text

Personal Protective Equipment (PPE)-*Mark all PPE that will be used in project.*

Personal Protective Equipment	Lab Procedures	Human/Animal Procedures	Plant Procedures
Laboratory Coat	☒	☐	☐
Gloves	☒	☐	☐
Safety Glasses/Goggles	☒	☐	☐
Face Shield	☐	☐	☐
Surgical Mask	☐	☐	☐
Respirator*	☐	☐	☐
Disposable Lab Coat	☒	☐	☐
Booties	☐	☐	☐
Hair/Head Cover	☐	☐	☐
Other (specify)			

**All respirators, including N-95 respirators, require medical clearance and fit testing to confirm the appropriate fit and protection. Additional information on the Respirator Program can be found on the website or by contacting the Safety Office (231.7759)*

Principal Investigator Assurance

By submitting this application, I agree to:

☒ Conduct this project in compliance with all policies and directives of North Dakota State University, as well as all applicable US laws.

☒ Be adequately trained in good microbiological techniques

☒ Instruct and train laboratory and research staff in the practices and techniques required to ensure safety and the procedures for dealing with accidents and releases

☒ Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or recommended (e.g. vaccinations or serum collection)

☒ Supervise laboratory and research staff to ensure that required safety practices and techniques are followed.

☒ Correct work errors or conditions that may result in the release of recombinant or synthetic nucleic acid materials or pathogens

☒ Ensure all laboratory and research staff have access to the protocols describing the potential biohazards and necessary precautions

☒ Maintain the integrity of physical containment (e.g. biological safety cabinets) and biological containment (e.g. host-vector system that preclude survival of the agent outside the lab).

☒ Maintain a current inventory of infectious agents and toxins on file.

The information within this application is accurate to the best of my knowledge.

Principal Investigator Signature Dharmakeerthi Nawarathna Digitally signed by Dharmakeerthi Nawarathna Date: 2019.08.02 14:50:09 -05'00'

The signature below certifies acknowledgement that the research outlined in this application is in keeping with standards set by your department/unit, all NDSU policies and that facility, equipment and personnel are appropriately committed to this project.

Chair, Head, Director or Dean Signature Benjamin D. Braaten Digitally signed by Benjamin D. Braaten Date: 2019.08.05 09:13:52 -05'00'

Institutional Biosafety Committee

The IBC has reviewed and approved this protocol.

IBC Chair GEORGE YOCUM Digitally signed by GEORGE YOCUM Date: 2019.08.13 09:40:49 -05'00' ***Date***

Submit by Email