

Overseas Practice on (Field Epidemiology • Collaborative

Research) report form (For Student)

2016/02/10

(Year/Month/Day)

Name	Zhifu Shan
Laboratory	Veterinary Hygiene
Year (Grade)	D3
Place of practice	TSE/Prion section of Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana 59840 USA
Period of practice	2016/01/11-2016/01/29
Purpose	To learn the skill of RT-QulC analysis

Summary of activities (about 800 words, provide photos, tables and figures that clearly show the activities during the period)

Background and purpose

The Rocky Mountain Laboratories (RML) is located in Hamilton, Montana, a small but thriving community nestled between the Bitterroot and Sapphire Mountains (fig 1.), which is a part of National Institute of Allergy & Infectious Disease (NIAID), National Institutes of Health (NIH) of USA. RML plays a key role in the nation's health and well-being by focusing its talent and resources on the infectious disease such as influenza, prion diseases, and antibiotic-resistant bacteria.

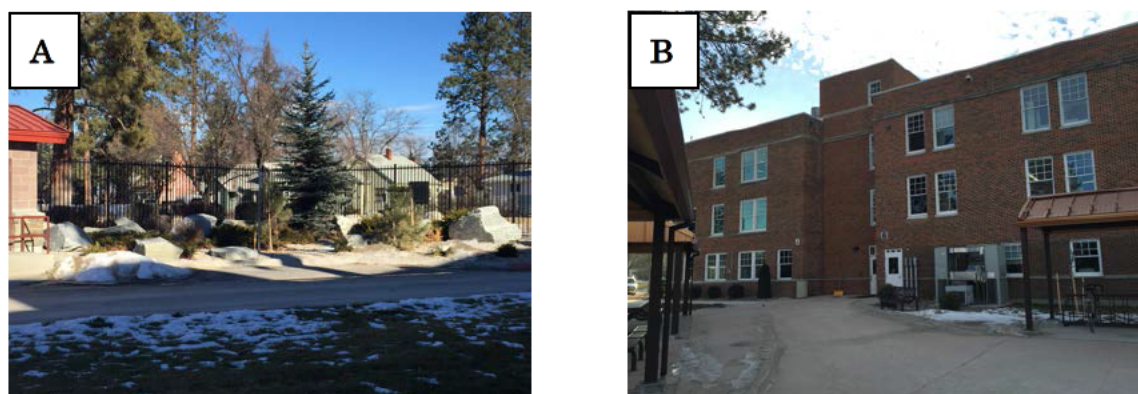


Fig 1. The front door of Rocky Mountain Laboratories (A) and the building where prion laboratories located in (B).

The scientific programs at RML are organized into five laboratories, this time I went to the TSE/Prion biochemistry section of the of Persistent Viral diseases Laboratory, which is one of the most famous laboratories in the field of prion research all over the world. This laboratory

focuses primary on dissecting the structure of prions as well as the diagnosis and treatment of prion diseases. Currently, they and others have developed ultrasensitive real time quaking-induced conversion (RT-QuIC) assays for the rapid diagnosis and detection of a wide variety of prions in many types of samples. My lab has been collaborating with RML in many aspects of prion diseases. Regarding to RT-QuIC studies, my lab is collaborating the use of immunoprecipitation to enhance the sensitivity of RT-QuIC reaction. Now I am doing my Ph.D. research on the therapeutics of prion diseases. High sensitive diagnosis method by which diagnosis can be made in an earlier clinical stage is required to enhance the efficacy of therapeutics. Therefore, I am interested in the advanced diagnosis technology of RT-QuIC and planning to go to the TSE/Prion section of RML to learn the skill of RT-QuIC analysis. As a collaborative research, I will attempt to find conditions under which we can distinguish different prion strains in animals by RT-QuIC analysis.

Activities

As a national institute of USA, RML has strict management for the performance of biological procedures and the disposal of infectious waste. Before I start to do experiments there, I learned their basic standard operating procedures for prion BSL 2 or 3 laboratories from some documents and videos.

The first week I learned the whole progress of the RT-QuIC analysis and began with the expression and purification of recombinant prion protein (rPrP). The type and quality of the rPrP is very important and determine the sensitivity of the RT-QuIC analysis. Primarily, the bacterial which contains the plasmid of rPrP was cultured and the rPrP was expressed in the inclusion bodies. Then the inclusion bodies were isolated and the rPrP was purified by Nickel-NTA. After obtaining the rPrP, I learned how to do RT-QuIC assay to detect prion-infected brain tissues with rPrP.

The second week I tried to use mouse rPrP to detect the sensitivity of L-BSE from bovine brain by RT-QuIC. The reason I choose L-BSE strain is that the similar detection was tried in our laboratory and met some difficulties in the detection of low dilution of brain homogenate. I followed the protocol they previously do here and get the ideal result, and found one of the difference between our experiment and theirs is the amount of L-BSE brain homogenate used as seeds. In our case, we use more amount of L-BSE brain homogenate as seeds which may inhibit the progress of transform from rPrP to infectious PrP in low dilution of the seeds.

The last week I prepared my own buffers for the RT-QuIC analysis and checked them with hamster rPrP and prion strain 263K as seed. After confirming the effectivity of my buffers, I

used both mouse rPrP and hamster rPrP as substrates and compared the sensitivity of L-BSE from bovine brain by RT-QuIC. (Fig 2.)The result showed similar sensitivity in mouse and hamster rPrP (Fig 3.), but mouse rPrP seems more easy to react in low dilution of brain homogenate. What's more, I talked with the researchers there on their new achievement that different BSE strains can be discriminated by RT-QuIC assay. Actually, the recombinant bank vole PrP was used as substrates for the RT-QuIC assay. It is a universally effective substrate for multiple prion strains from multiple species. According to the different starting point of reaction in different prion strains, we can discriminate the strains by RT-QuIC assay.

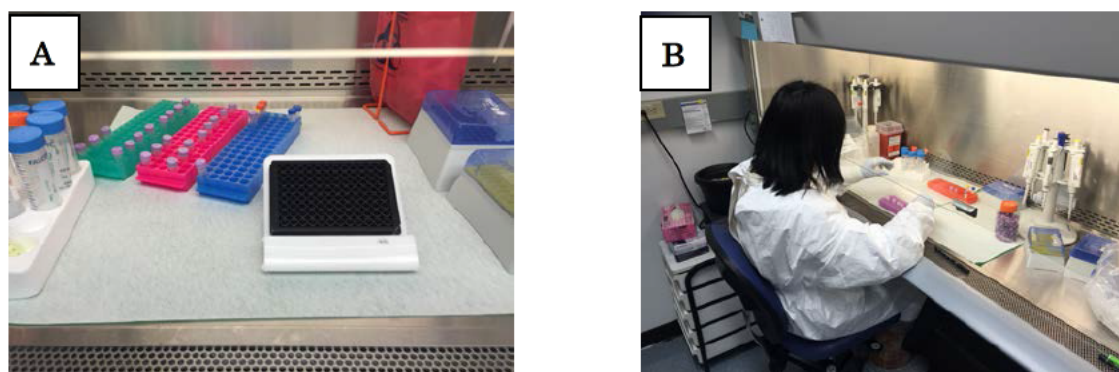


Fig 2. Practice of RT-QuIC analysis

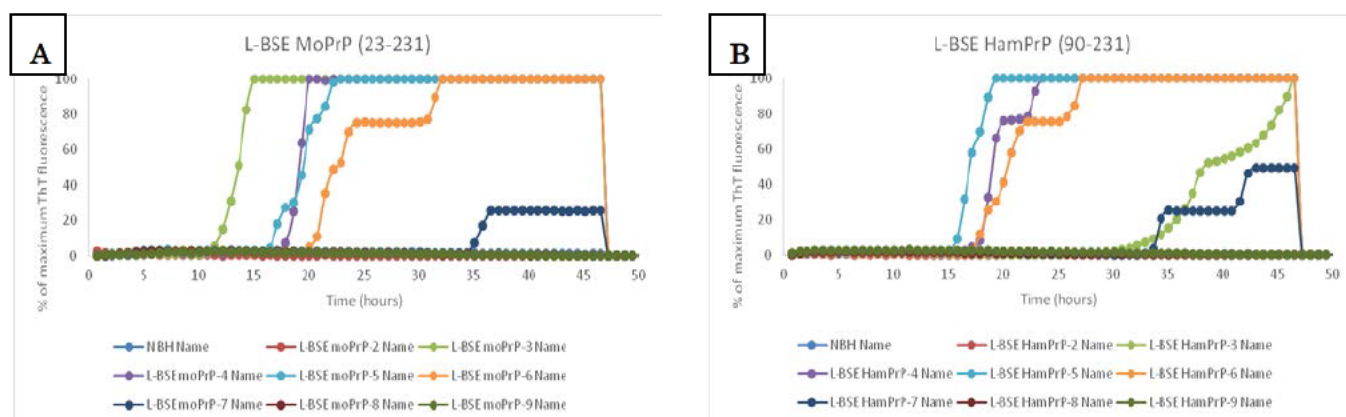


Fig 3. The result of RT-QuIC detection of L-BSE from brain homogenate using mouse and hamster rPrP.

After three weeks study in RML, I learned how to diagnose the prion infection from different animals brain homogenate by RT-QuIC analysis and they also show me some application of RT-QuIC assay they have been found and trying to find. This collaboration trip offered me a good chance for communication and thinking, from those, I learned the new skill and also some good habit for doing research. For example, they attach great importance to the analysis of the data on time, which widen the notion and support for the next step of experiment.

Finally, I want to thank professor Horiuchi and leading program give me this chance to go to RML, it is a memorable experiences and will be helpful me to study in the future.

(Field Epidemiology • Collaborative Research) Evaluation by supervisor

Institution • Official title • Name	Motohiro Horiuchi	印
My lab has been collaborating with Dr. Coughy's lab for a long time and this time, Ms. Shan Zhifu She accomplished the primary purpose of her visit; to learn basic skill of RT-QuIC assay. She also had a great experience through interacting scientist at RML not only in science but also cultures.		

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