Real-Time Quaking Induced Conversion Assay for Prion Seeding

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Introduction

Prions are transmittable pathogens that cause an abnormal folding of a brain protein in both humans and animals. Infection results in brain damage and is fatal. Some examples of these neurodegenerative diseases are Scrapie, Bovine Spongiform Encephalopathy, and Creutzfeldt-Jakob Disease. Previously, prions were studied using lengthy bioassays where infected animals were studied over long periods of time (1-6 months). This was both time consuming and costly to maintain the infected animal. Researchers at Rocky Mountain Laboratories in Hamilton, Montana have developed a prion seeding assay called Real-Time Quaking Induced Conversion Assay (RT-QuIC) that gives end point quantitation for measuring the levels of prions in infected samples. This assay is faster and yields higher throughput compared to previous methods. The assay can be completed in as few as 20 hours and is as sensitive, if not more so, as whole animal models.

Assay Principle

Combining parts of the original Quaking Induced Conversion (QuIC) assay and the amyloid seeding assay (ASA), the RT-QuIC assay is used to estimate the relative amount of prion seeding. The assay measures serial dilutions of samples, statistically estimating the seeding dose (SD). Very small amounts of infectious prions are added to normal prion protein to seed or cause the misfolding of the prion proteins as seen in the disease. The assay is quantitated by measuring serial dilutions of the samples and determining the loss of seeding activity, which is the end point dilution.

The fluorescent dye thioflavin T (ThT) is used as a prion seeding marker. When ThT is added to recombinant prion proteins, it becomes incorporated when polymerization occurs causing an increase in fluorescence over time.

BMG LABTECH's Omega series of readers have the ability to shake and incubate microplates over long periods of time. A POLARstar Omega was used to measure RT-QuIC samples every 15 minutes for 20-68 hours while alternately shaking and resting for a minute.

Results and Discussion

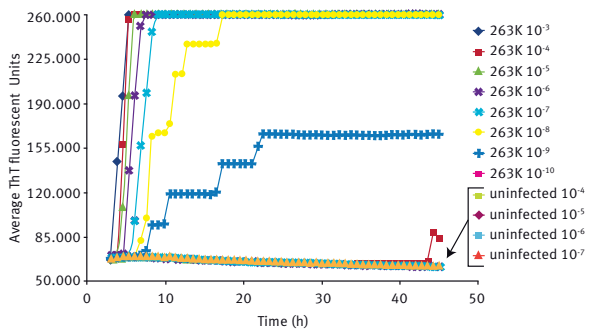

Fig. 1: RT-QuIC sensitivity: analysis of dilutions of a scrapie hamster brain homogenate stock 263K 80 - 85 dags post infection (DPI).

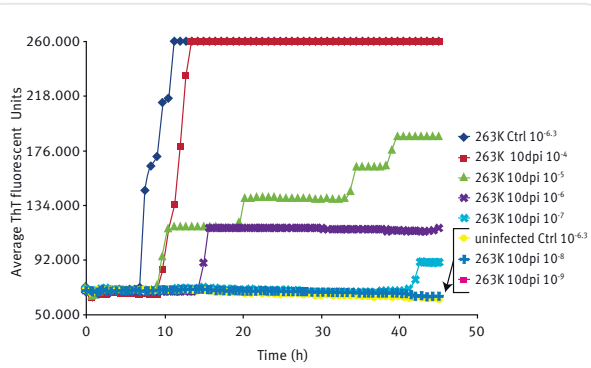

Fig. 2: RT-QuIC end-point dilution analysis of three 263K-inoculated preclinical 10 days post injection hamster BHs.

The SD50/gram of tissue for the 85 DPI samples (10E12) was higher than the 10 DPI (10E8.2) because it had a longer time for onset.

Conclusion

Prion seeding can be measured faster and in a higher throughput using the RT-QuIC assay and a microplate reader. Some of the transmissible spongiform encephalopathies that have been shown to work using RT-QuIC include hamster and sheep scrapie, deer chronic wasting disease, Creutzfeldt-Jakob Disease (CJD), and Bovine Spongiform Encephalopathy (BSE). The Omega series of plate readers from BMG LABTECH are both functional and robust to withstand the many days of shaking at high speeds required for this assay.