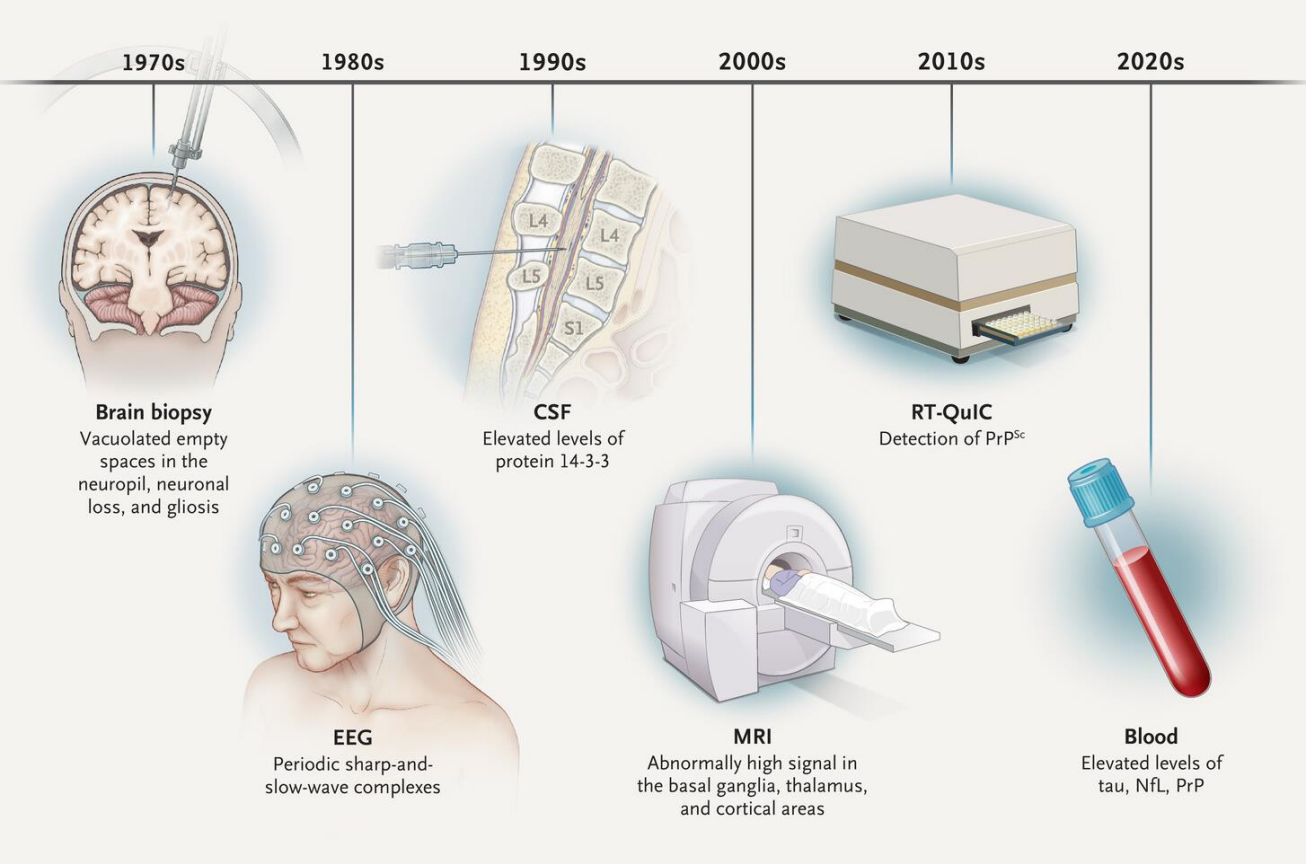
Development of a blood & urine prion diagnostic test for human prion diseases using Real Time QuIC assays

Presenting author: **Sarah Vascellari, PhD**
Department of Biomedical Sciences, University of Cagliari, Italy

CJD Foundation Family Conference
Washington, DC July 19-21, 2024

Study participants: **Sarah Vascellari,** University of Cagliari, Italy; **Christina D. Orrù**, RML, NIH, MT, USA; **Byron Caughey**, RML, NIH, MT, USA; **Franco Cardone**, ISS, Italy; **Pierluigi Gambetti,** Case Western Reserve University, USA; **Stephane Haik** Sorbonne University, France; **Gianluigi Zanusso**, University of Verona, Italy; **Larisa Cervenakova**; **Aldo Manzin**, University of Cagliari, Italy

Diagnosis of sporadic CJD



Definite:
Progressive neuropsychiatric syndrome and neuropathological or immunocytochemical, or biochemical confirmation

Probable:
I + two of II and typical EEG*
or
I + two of II and typical brain MRI†
or
I + two of II and positive CSF 14-3-3
or
progressive neuropsychiatric syndrome and positive RT-QuIC in CSF or other tissues
+ exclusion of other causes in complete diagnostic workup

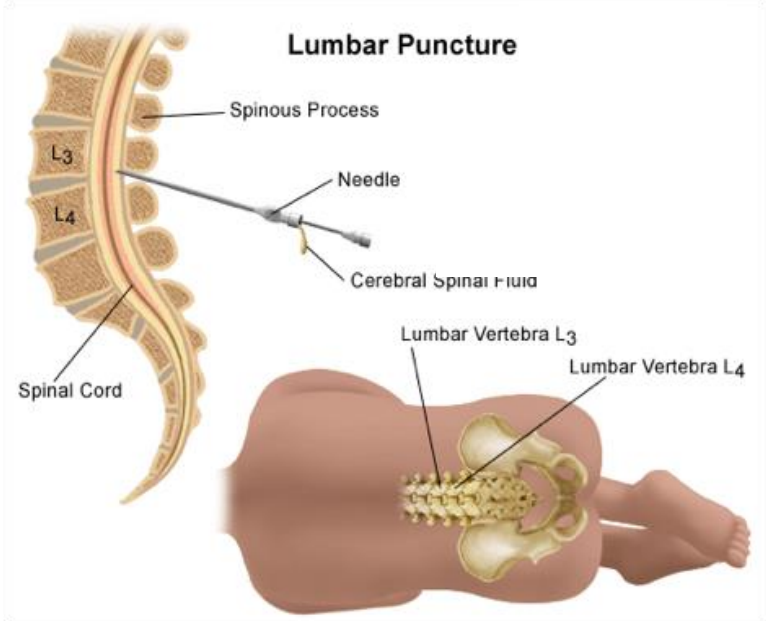
Possible:
I + two of II + duration <2 years

I
Rapidly progressive cognitive impairment

II
A. Myoclonus
B. Visual or cerebellar disturbance
C. Pyramidal or extrapyramidal signs
D. Akinetic mutism

Hermann P et al The Lancet of Neurology 2021; Inga Zerr N Engl J Med 2022

RT-QuIC assay for diagnosis of sCJD



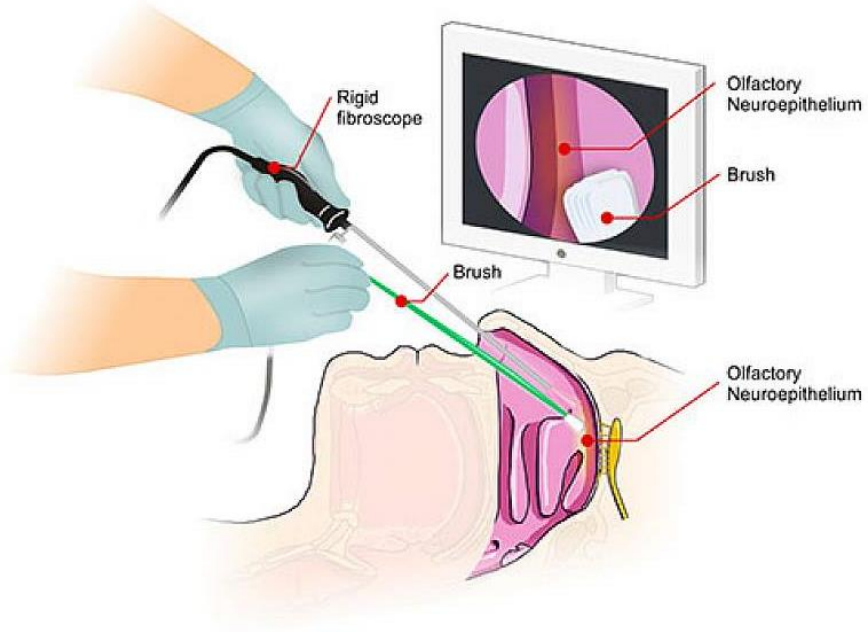
Rapid and Sensitive RT-QuIC Detection of Human Creutzfeldt-Jakob Disease Using Cerebrospinal Fluid

Christina D. Orrú,[a] Bradley R. Groveman,[a] Andrew G. Hughson,[a] Gianluigi Zanusso,[b] Michael B. Coulthart,[c] Byron Caughey[a]

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA[a]; Department of Neurological and Movement Sciences, University of Verona, Verona, Italy[b]; Canadian CJD Surveillance System, Public Health Agency of Canada, Ottawa, Ontario, Canada[c]

→ **overall sensitivity 70-100% and specificity 98-100% in CSF**

Nasal Brushing



The NEW ENGLAND JOURNAL of MEDICINE
2014;371:519-529

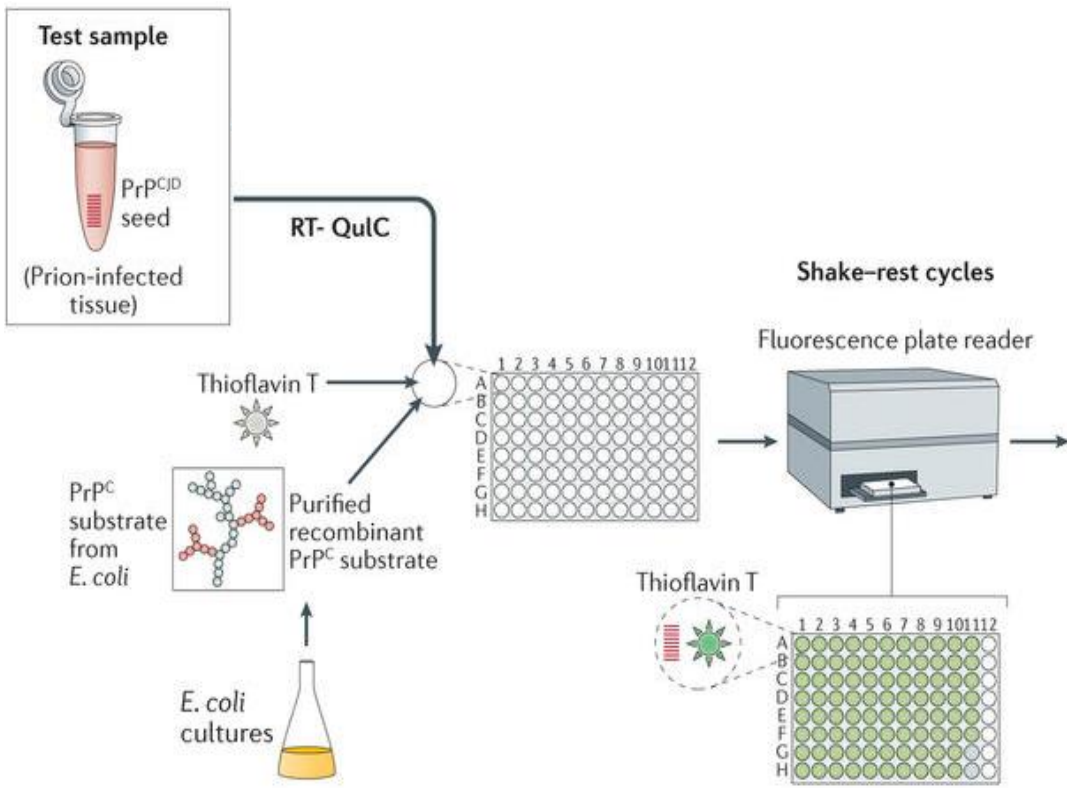
ORIGINAL ARTICLE

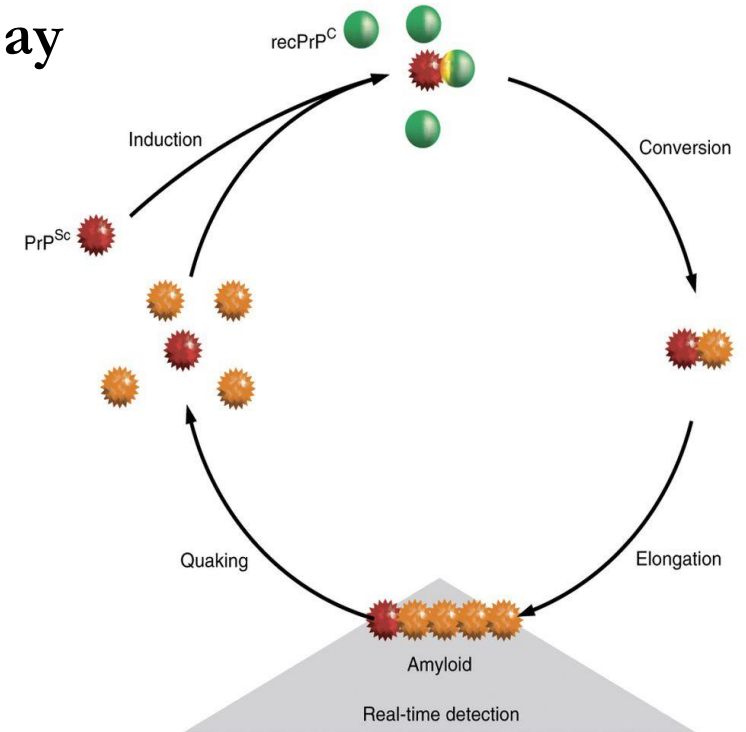
A Test for Creutzfeldt–Jakob Disease Using Nasal Brushings

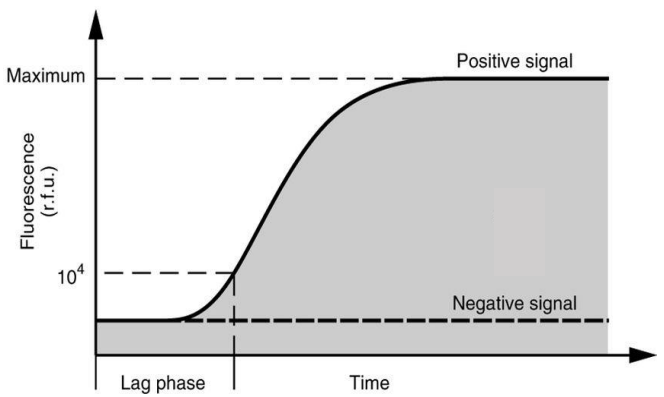
Christina D. Orrú, Ph.D., Matilde Bongianni, Ph.D., Giovanni Tonoli, M.D., Sergio Ferrari, M.D., Andrew G. Hughson, M.S., Bradley R. Groveman, Ph.D., Michele Fiorini, Ph.D., Maurizio Pocchiari, M.D., Salvatore Monaco, M.D., Byron Caughey, Ph.D., and Gianluigi Zanusso, M.D., Ph.D.

→ **97% sensitivity and 100% specificity in olfactory mucosa**

RT-QuIC assay



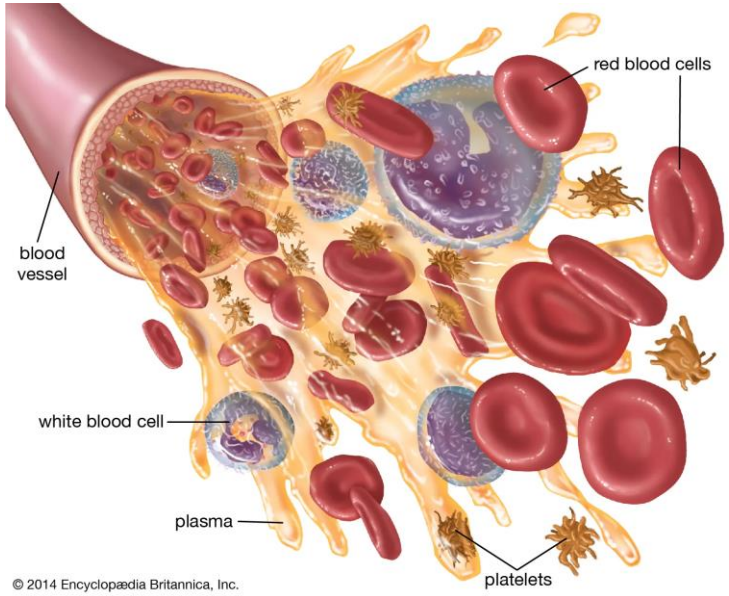




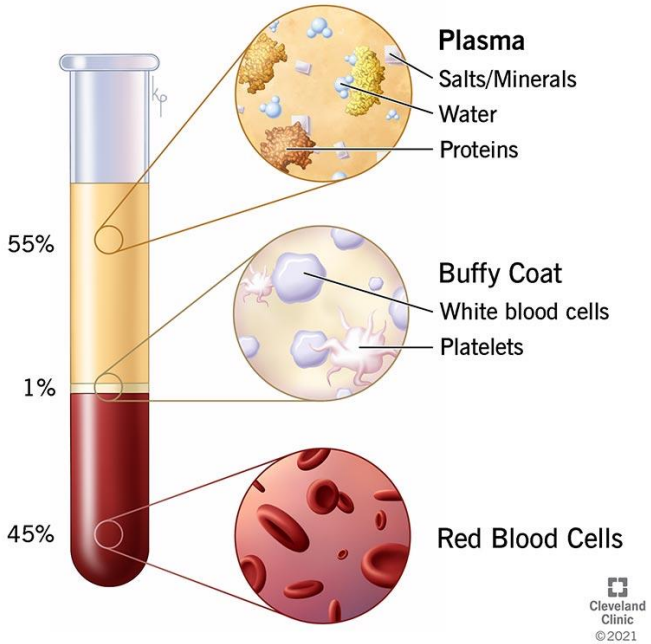
Nature Reviews | Neurology

Challenges in the development of a blood test for prion diseases

Whole blood sample



Fractioned sample



blood collection a non-invasive procedure

animal studies confirm the presence of prion infectivity in blood of sCJD-patients

Main issues

low levels of prions in blood

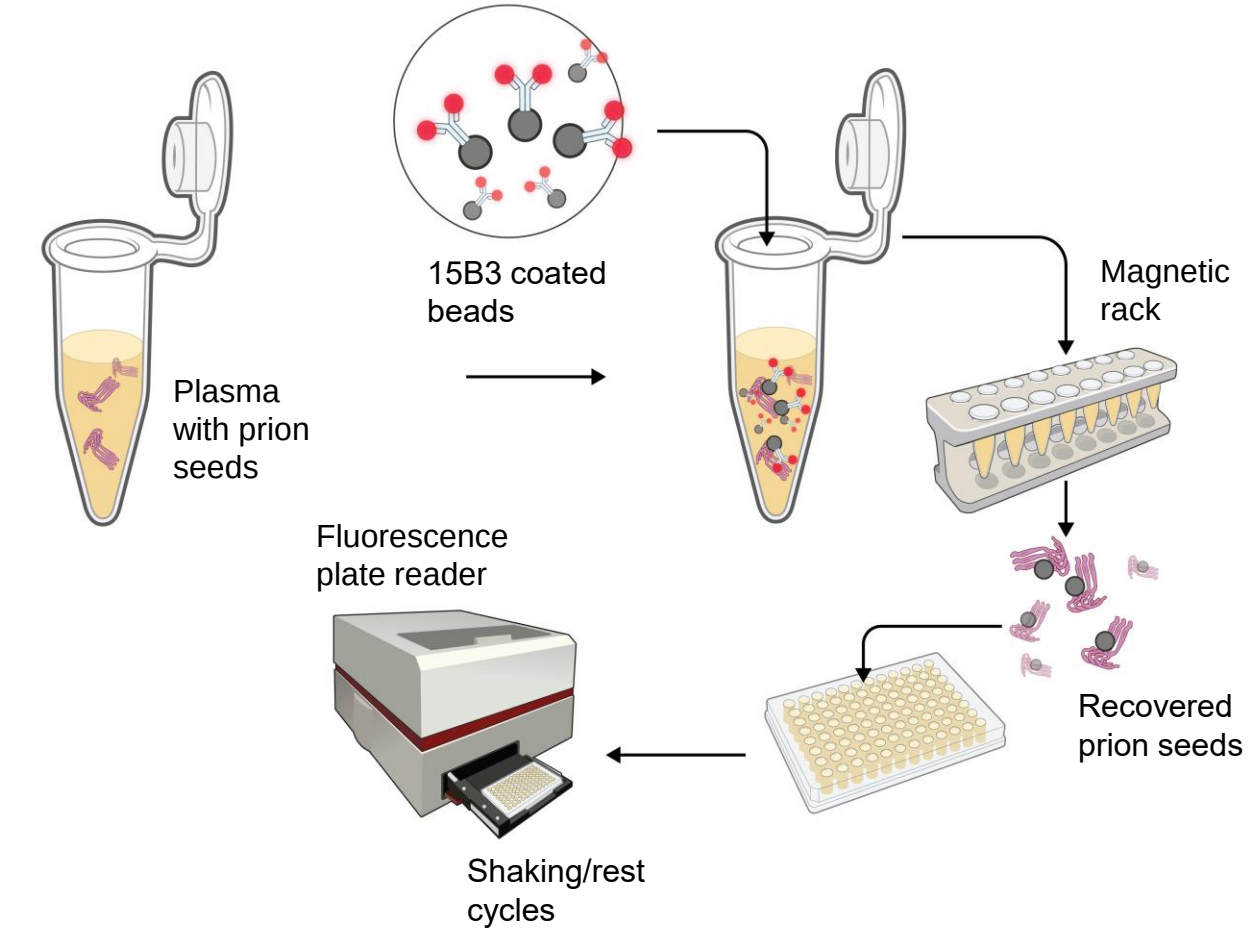
highest levels of infectivity are associated with leucocytes, whole blood, platelets, red cells & plasma

presence of RT-QuIC inhibitory components

Detection of prions in plasma from sCJD patients by eQuIC

Prion Disease Blood Test Using Immunoprecipitation and Improved Quaking-Induced Conversion

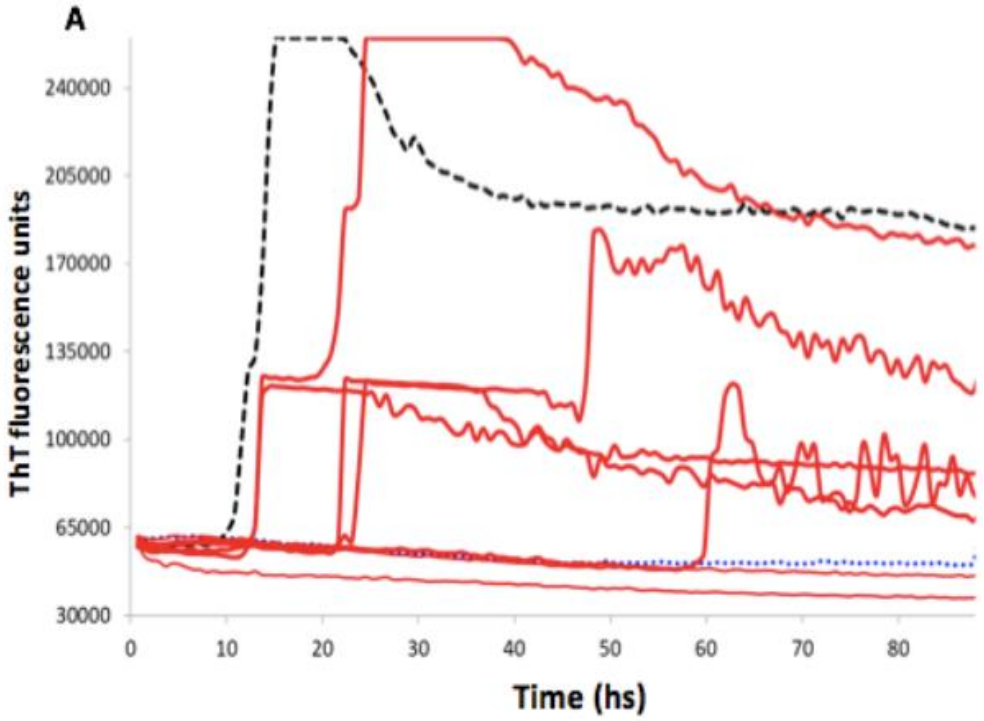
Christina D. Orrú,[a] Jason M. Wilham,[a] Lynne D. Raymond,[a] Franziska Kuhn,[b] Björn Schroeder,[b] Alex J. Raeber,[b] and Byron Caughey[a]
Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA,[a] and Prionics AG, Zurich, Switzerland[b]



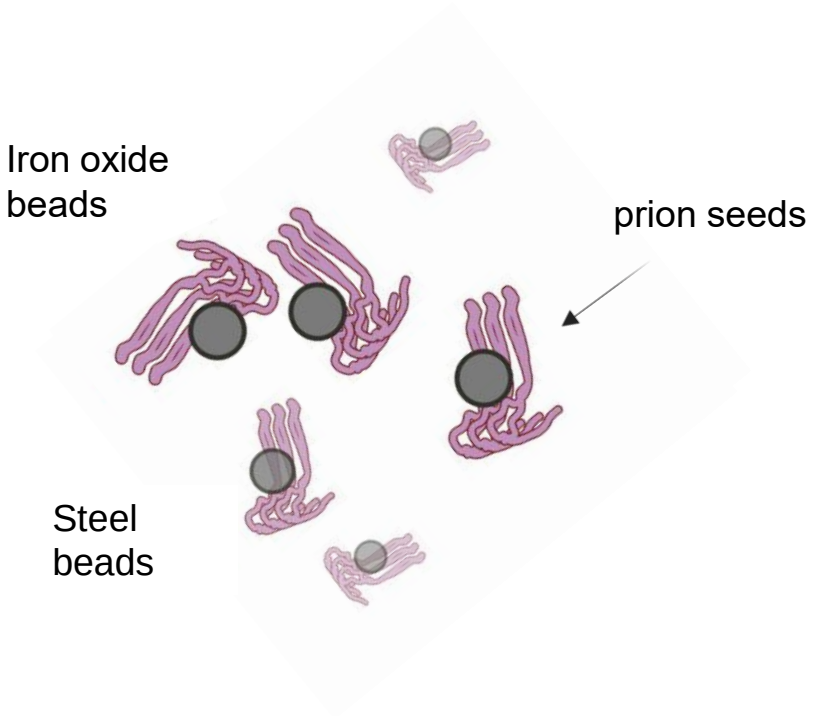
Credit: Ryan Kissinger, MSc, Visual Medical Arts, NIAID

eQuIC results testing sCJD blood plasma showed detection of PrP_{sCJD} in 5 out of 7 sCJD samples

eQuIC positive samples were subtype MM1 (n=3), MV1 (n=1) and VV1 (n=1) while the negatives were MV2 and MM1 subtype



Advanced approaches for prion capture and detection



Enhanced prion detection in biological samples by magnetic particle extraction and real-time quaking-induced conversion

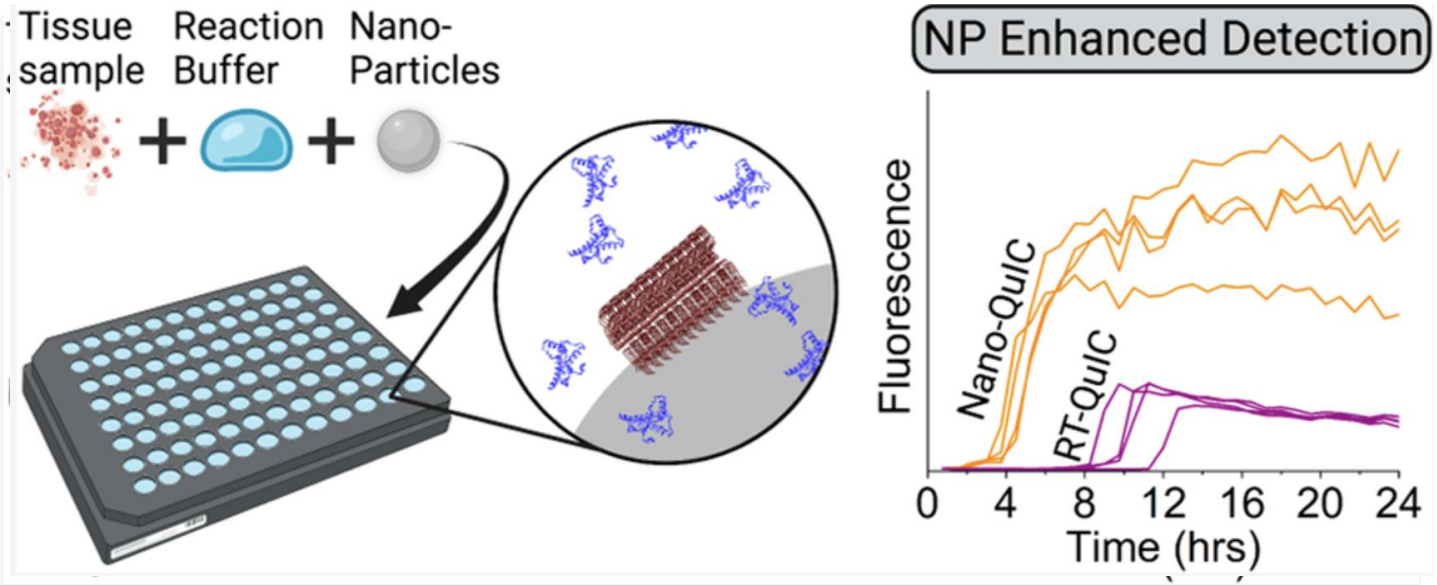
Nathaniel D. Denkers,† Davin M. Henderson,† Candace K. Mathiason and Edward A. Hoover

In vitro detection of haematogenous prions in white-tailed deer orally dosed with low concentrations of chronic wasting disease

Erin E. McNulty†, Amy V. Nalls†, Randy Xun, Nathaniel D. Denkers, Edward A. Hoover and Candace K. Mathiason*

Development of a sensitive real-time quaking-induced conversion (RT-QuIC) assay for application in prion-infected blood

Charlotte M. Thomas*, M. Khalid F. Salamat, Christopher de Wolf, Sandra McCutcheon, A. Richard Alejo Blanco, Jean C. Manson, Nora Hunter, E. Fiona Houston



Detection of prions in the urine of patients affected by sporadic Creutzfeldt–Jakob disease

Sandra Pritzkow[1], Frank Ramirez[1], Adam Lyon[1], Paul E. Schulz[1], Brian Appleby[2], Fabio Moda[3], Santiago Ramirez[1], Silvio Notari[2], Pierluigi Gambetti[2] & Claudio Soto[1]

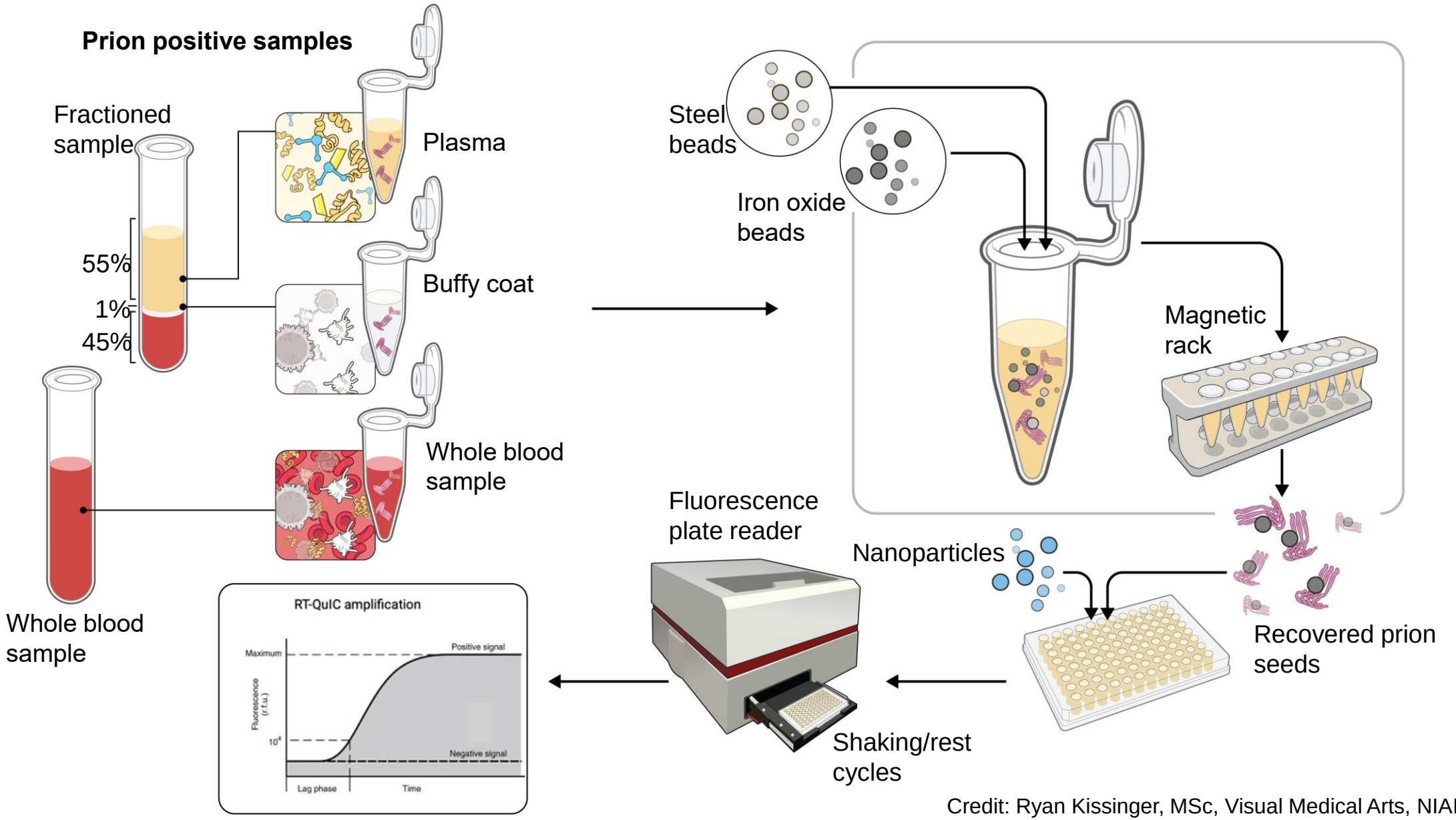
A Novel, Reliable and Highly Versatile Method to Evaluate Different Prion Decontamination Procedures

*Hasier Eraña[1,2†], Miguel Ángel Pérez-Castro[1†], Sandra García-Martínez[1,2], Jorge M. Charco[1,2], Rafael López-Moreno[1], Carlos M. Díaz-Domínguez[1], Tomás Barrio[1], Ezequiel González-Miranda[1,2] and Joaquín Castilla[1,3]**

Nanoparticle-Enhanced RT-QuIC (Nano-QuIC) Diagnostic Assay for Misfolded Proteins

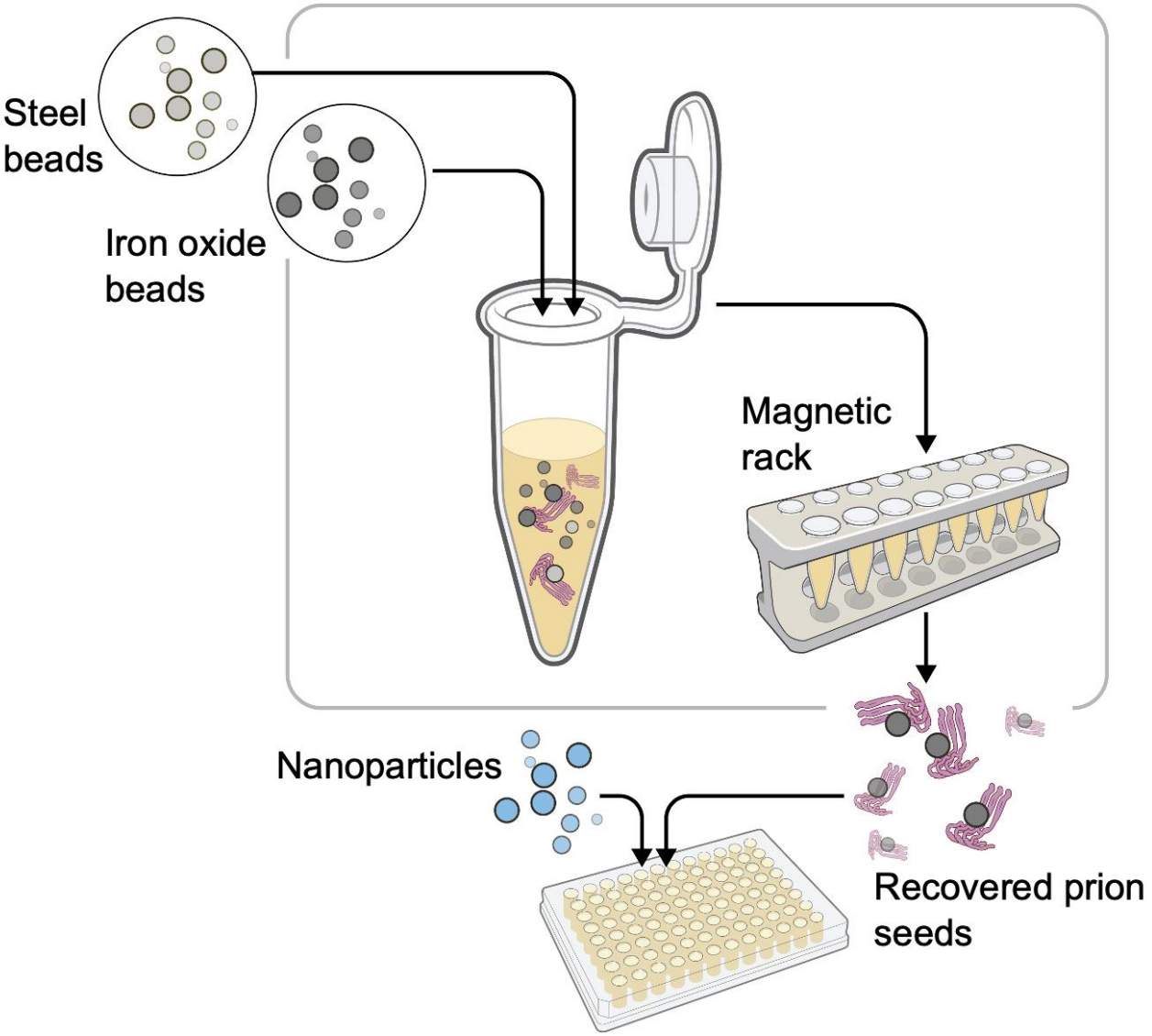
Peter R. Christenson, Manci Li, Gage Rowden, Peter A. Larsen,* and Sang-Hyun Oh*

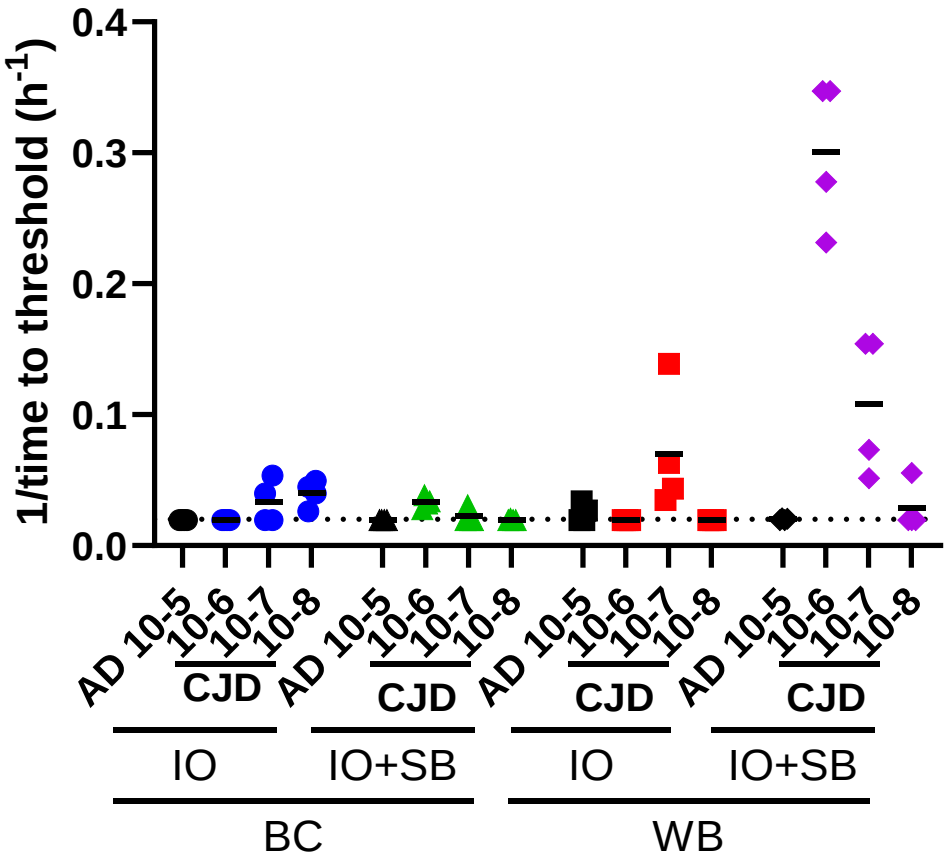
Metal beads prion-capture to isolate & concentrate prions from blood



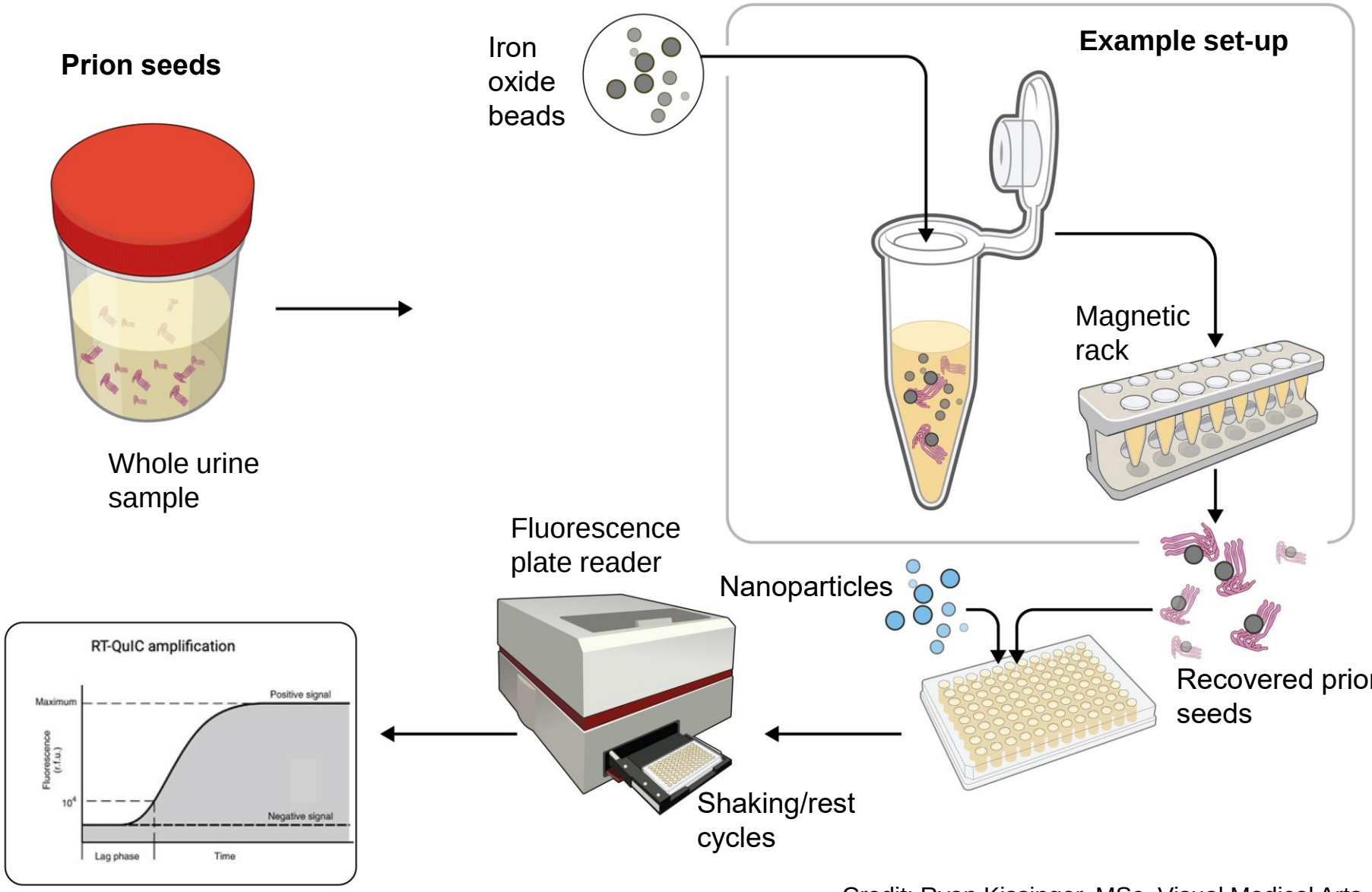
Credit: Ryan Kissinger, MSc, Visual Medical Arts, NIAID

Metal beads capture for prion detection from whole blood and buffy coat spiked with sCJD brains



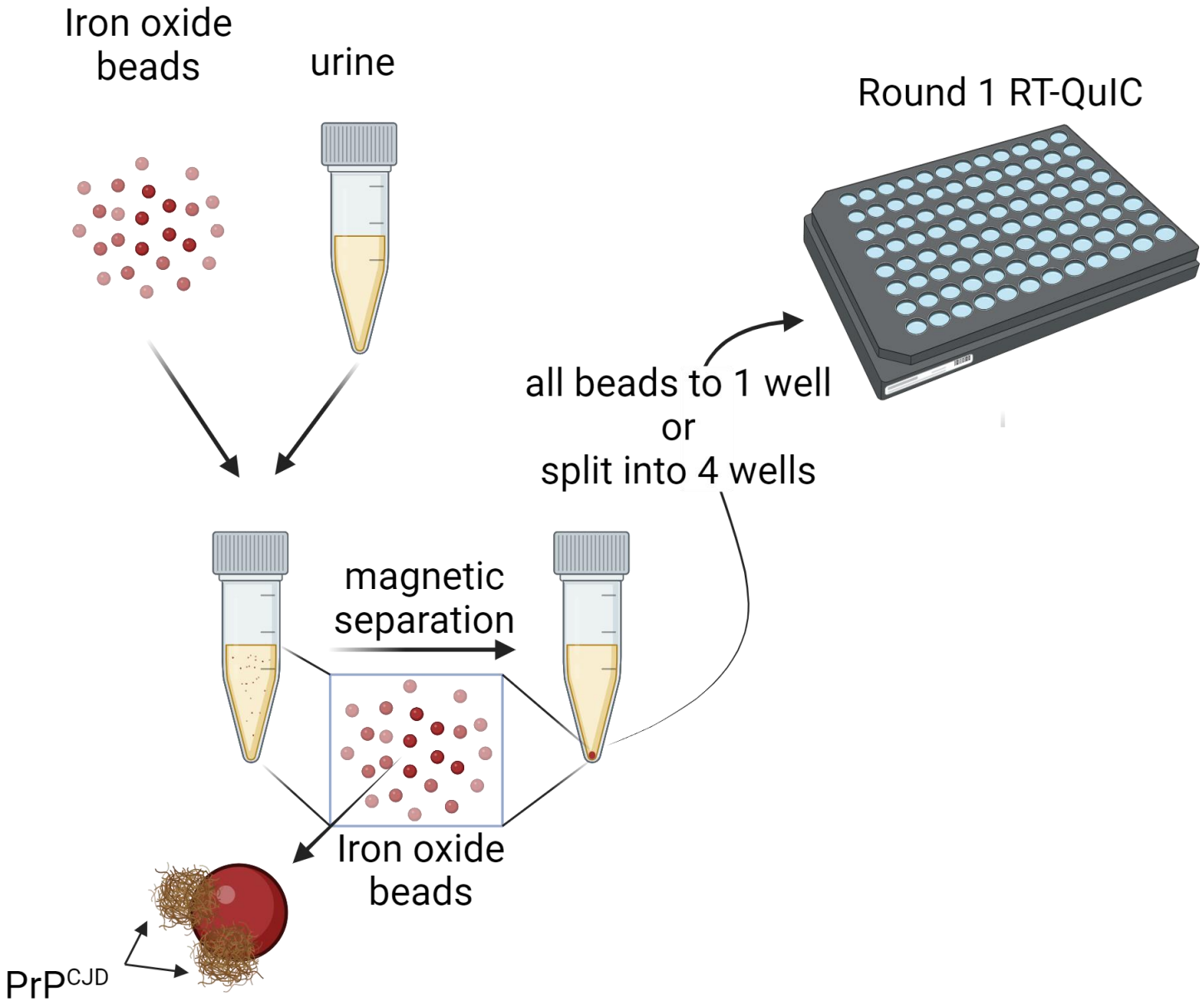


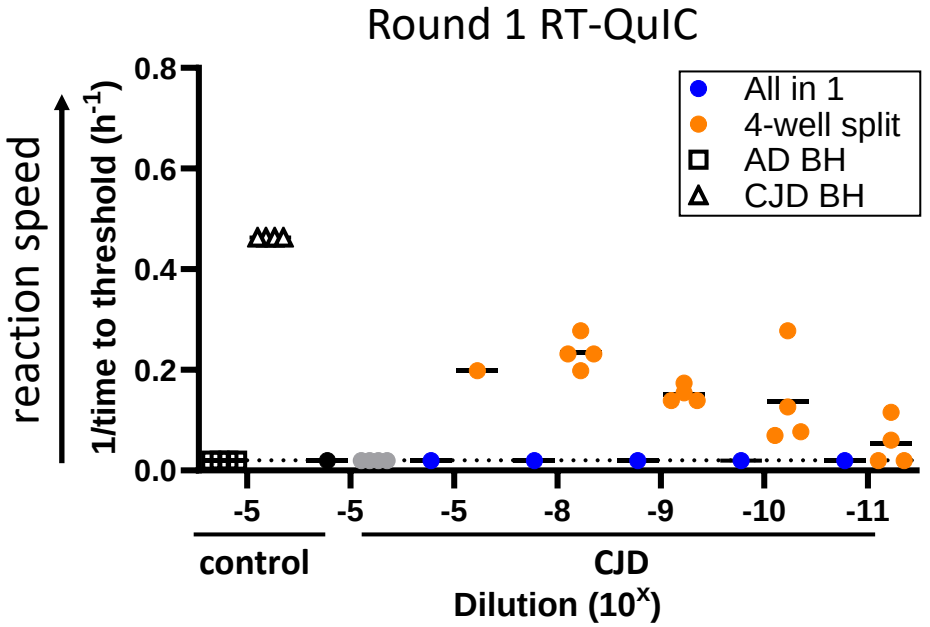
Iron oxide beads prion-capture to isolate & concentrate prions from urine



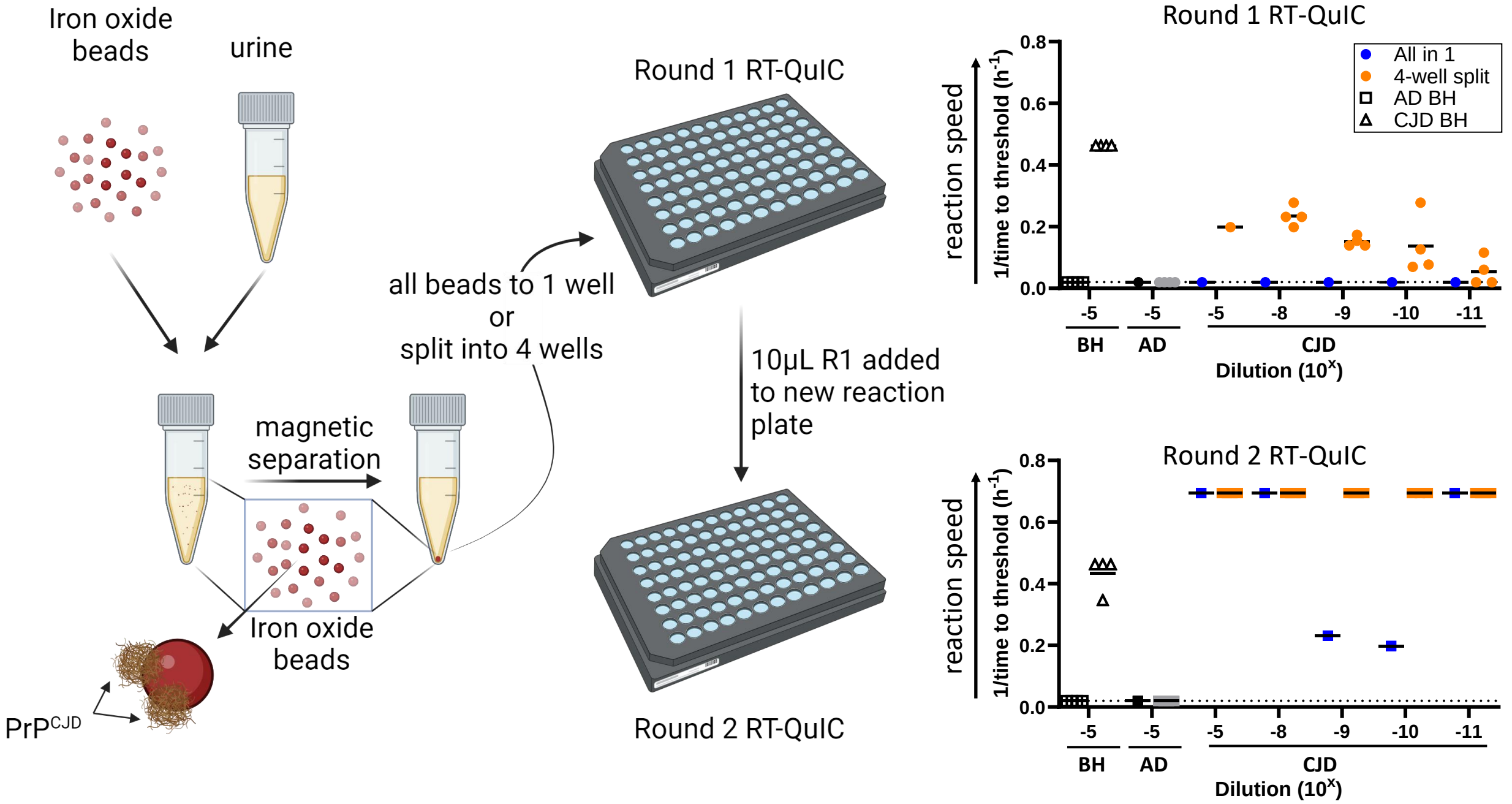
Credit: Ryan Kissinger, MSc, Visual Medical Arts, NIAID

IO capture for prion detection from urine spiked with sCJD brains

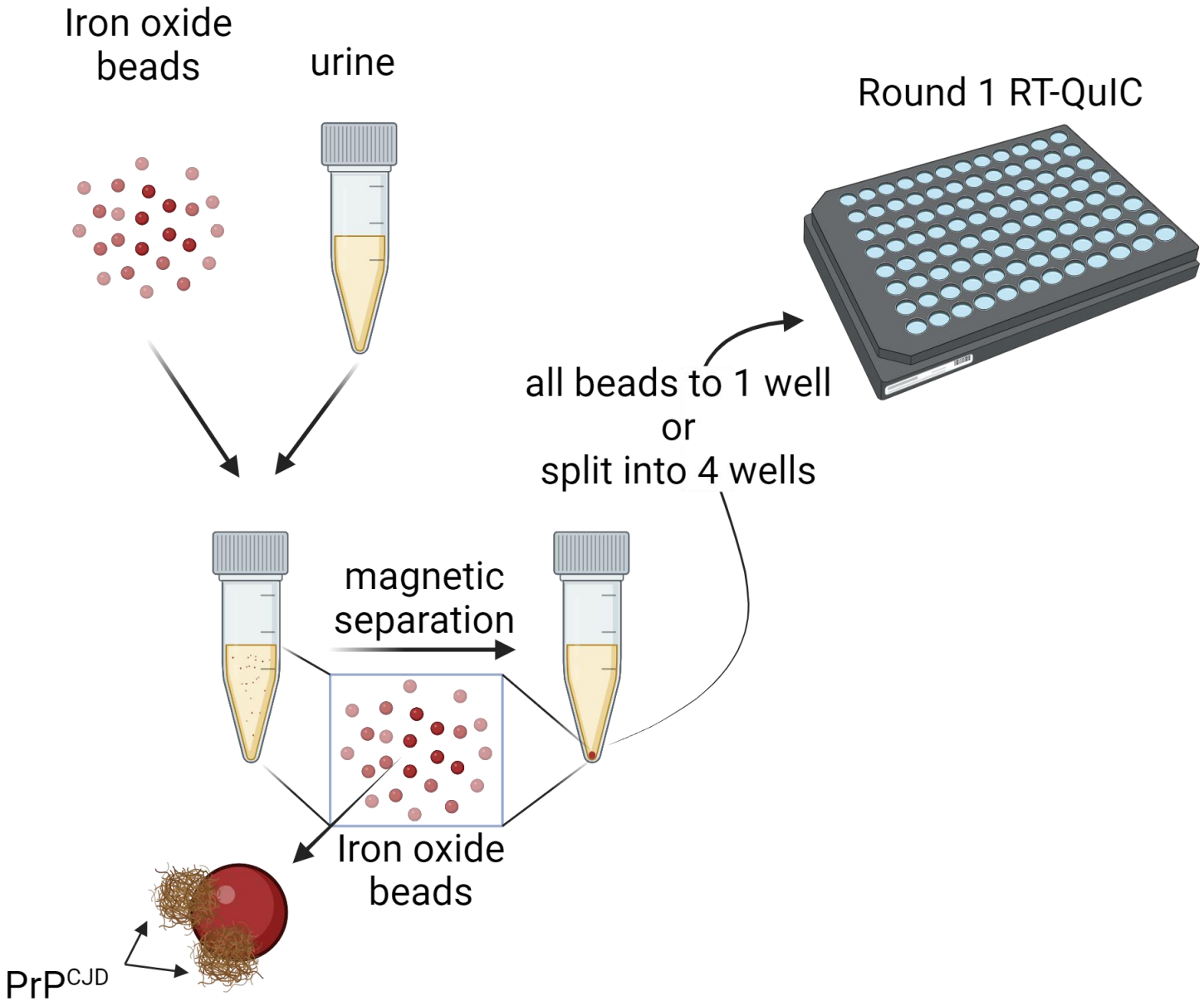


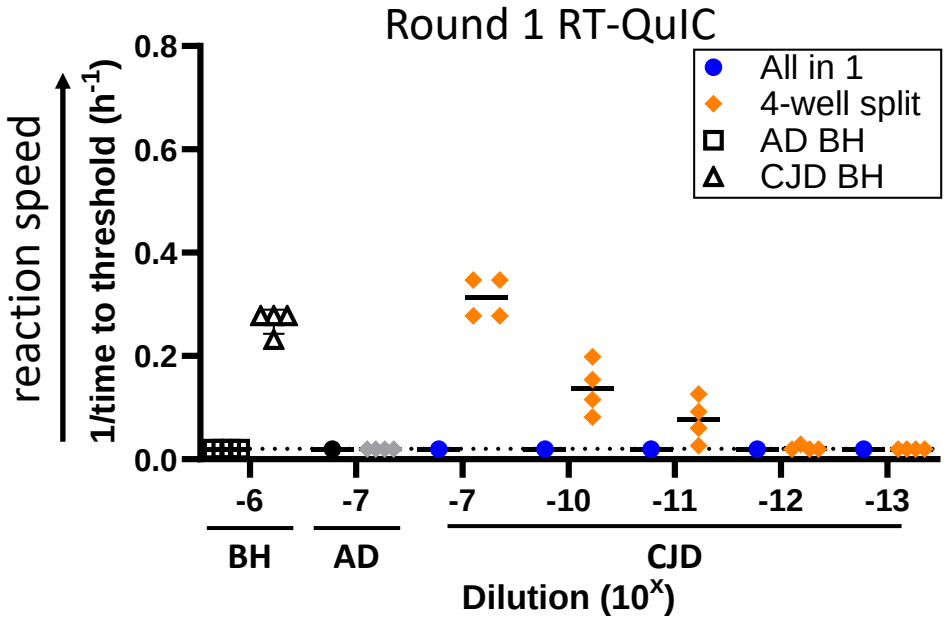


IO capture for prion detection from urine spiked with sCJD brains

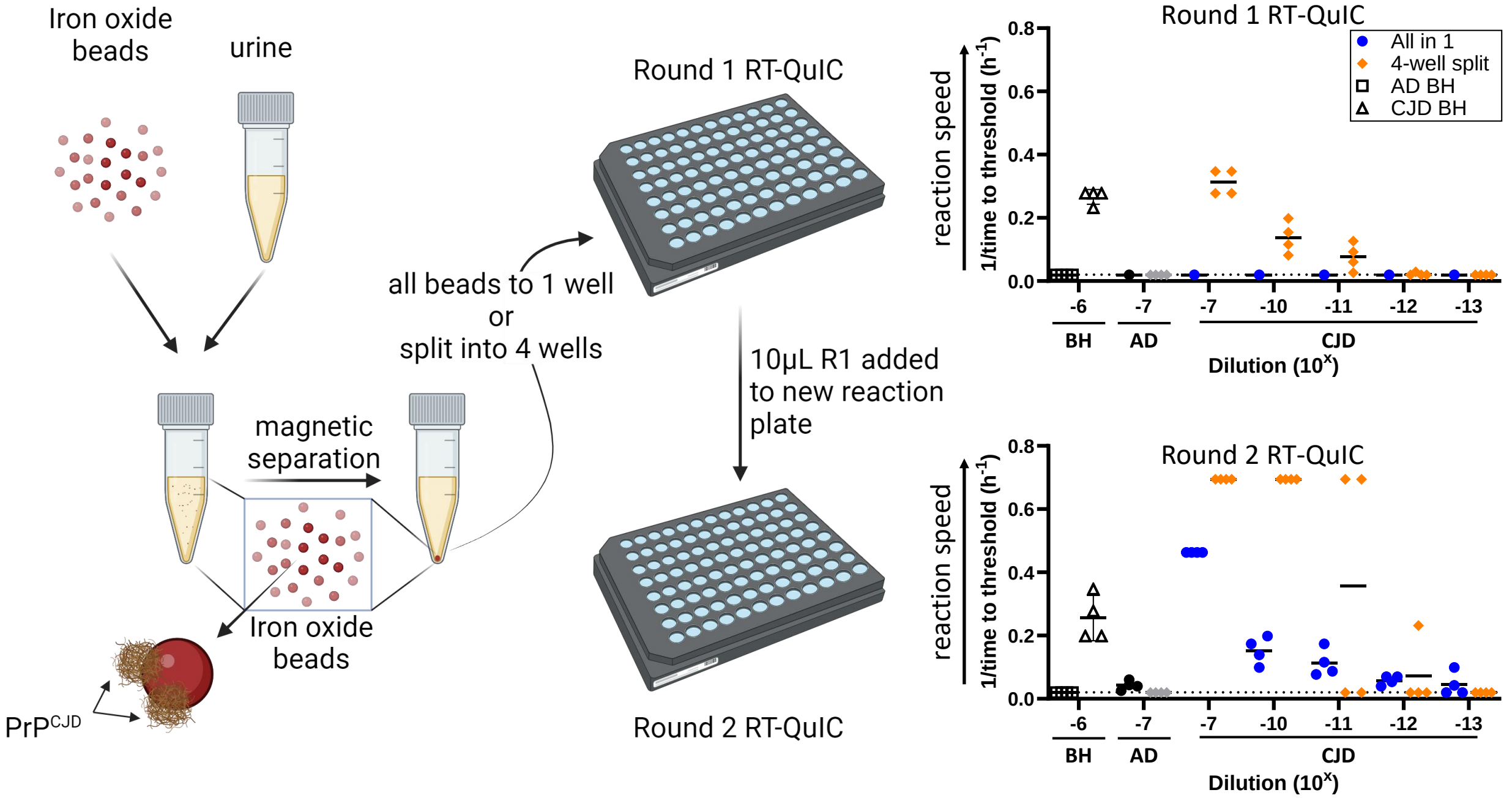


IO capture for prion detection from urine spiked with sCJD brains

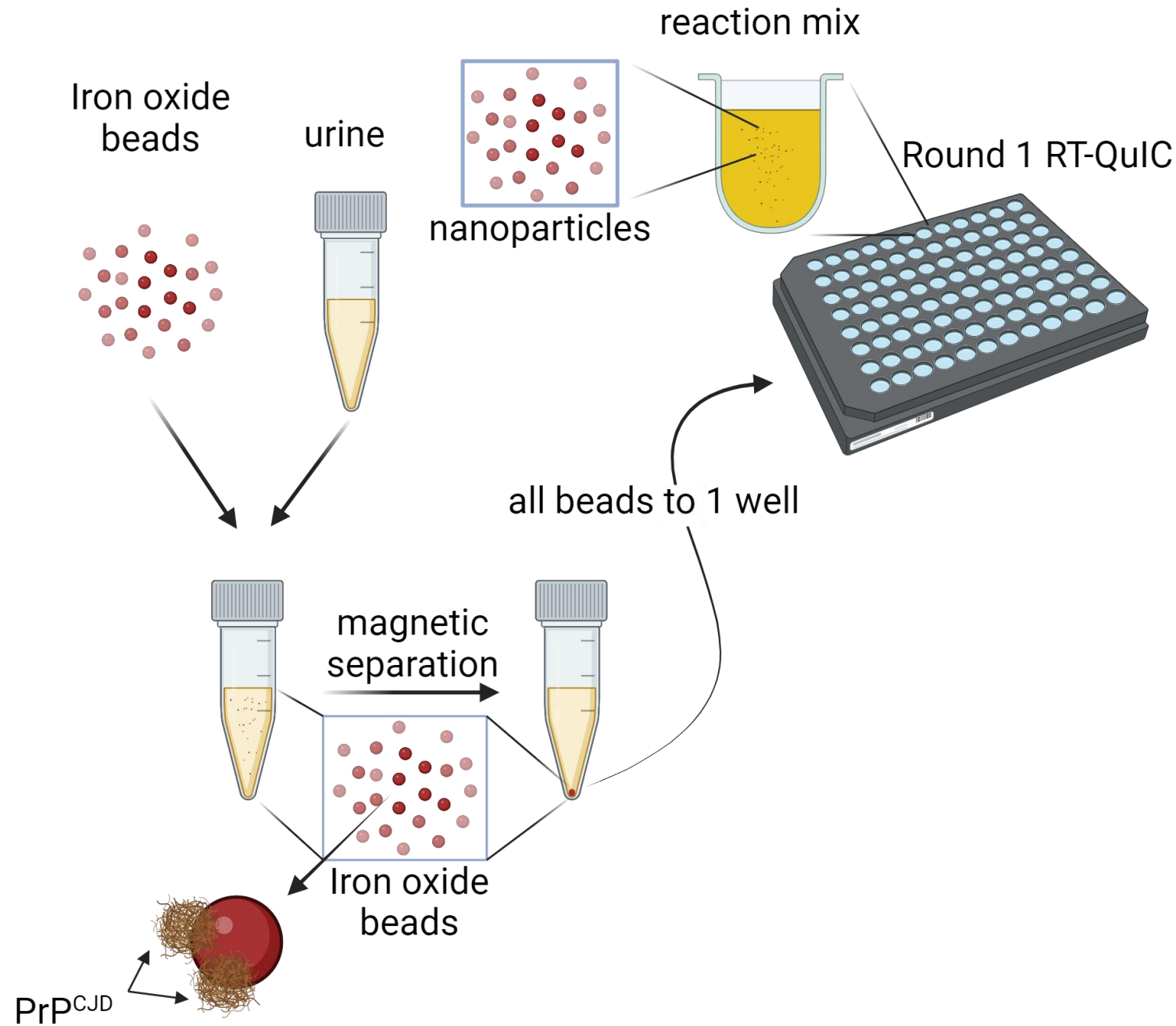


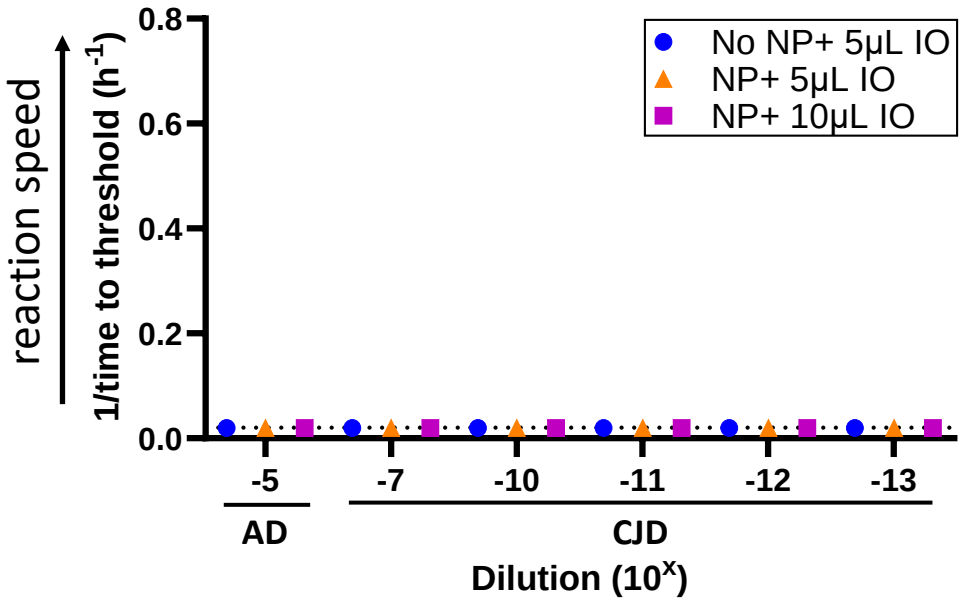


IO capture for prion detection from urine spiked with sCJD brains

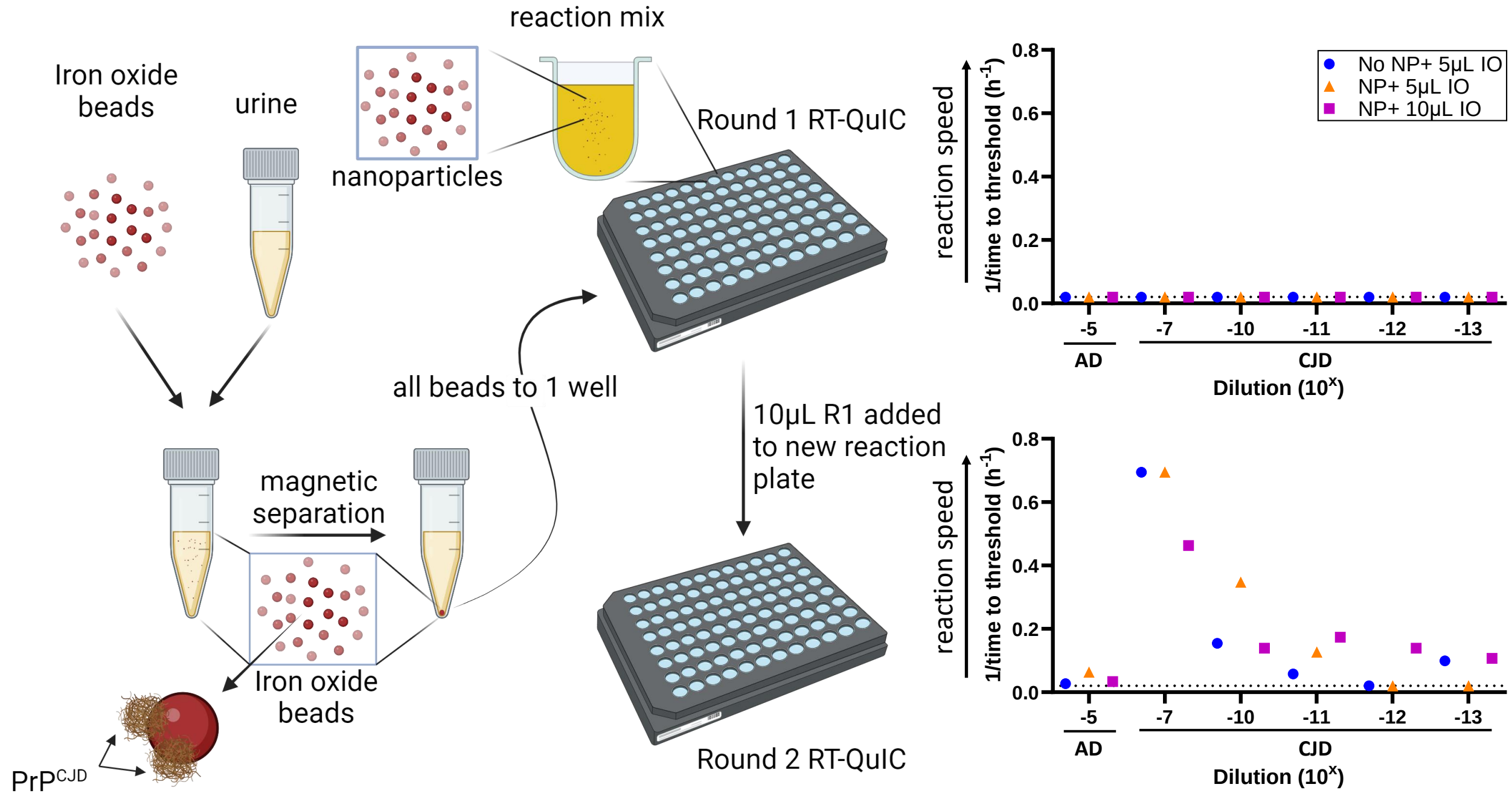


Nanoparticles with IO capture for prion detection from urine spiked with sCJD brains





Nanoparticles with IO capture for prion detection from urine spiked with sCJD brains



Presenting author
Dr. Sarah Vascellari

THE LABORATORY OF NEUROLOGICAL INFECTIONS & IMMUNITY

Development of a blood & urine prion diagnostic test for human prion diseases using Real Time QuIC assays

SUMMARY & CONCLUSIONS

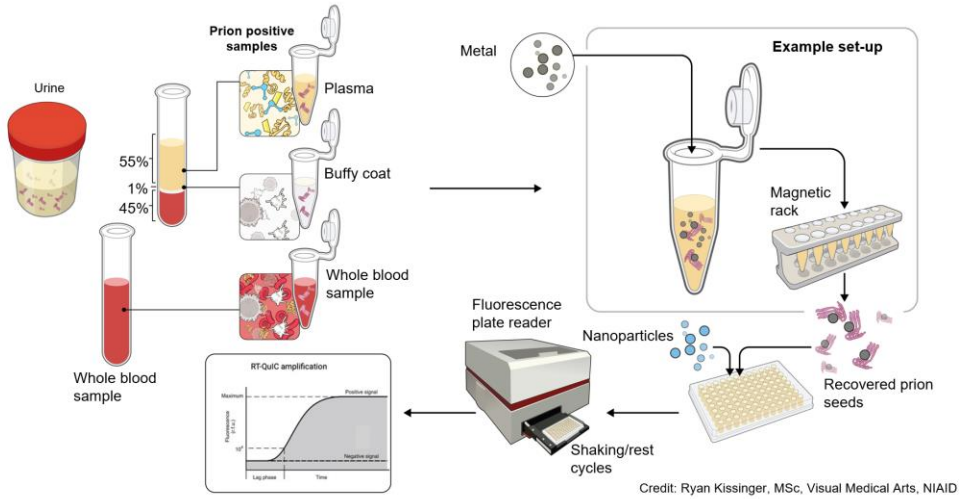
Cerebrospinal fluid RT-QuIC is a sensitive test for prion detection and is included in the diagnostic criteria for sporadic CJD

Several efforts have been made to use the RT-QuIC to test easily collectable samples such as blood & urine

Low prion concentration and the presence of RT-QuIC inhibitor components have been the main issues in using blood & urine for CJD diagnosis

We identified a promising approach based on metal beads prion-capture which allows to isolate & concentrate prions from blood and urine

Our current strategy may improve sporadic CJD prion detection in blood & urine samples



Study participants: **Sarah Vascellari,** University of Cagliari, Italy; **Christina D. Orrù**, RML, NIH, MT, USA; **Byron Caughey**, RML, NIH, MT, USA; **Franco Cardone**, ISS, Italy; **Pierluigi Gambetti,** Case Western Reserve University, USA; **Stephane Haik** Sorbonne University, France; **Gianluigi Zanusso**, University of Verona, Italy; **Larisa Cervenakova**; **Aldo Manzin**, University of Cagliari, Italy

Acknowledgments

Laboratory of neurological infections and immunity, NIAID, RML, NIH, USA
Christina D. Orrù
Byron Caughey

University of Cagliari
Aldo Manzin

Istituto Superiore di Sanità, Italy
Franco Cardone

University of Verona, Italy
Gianluigi Zanusso

Case Western Reserve University, USA
Pierluigi Gambetti

Sorbonne University, France
Stephane Haik

Larisa Cervenakova

Laboratory of neurological infections and immunity, NIAID, RML, NIH
Andrew Hughson

Visual Medical Arts, NIAID
Ryan Kissinger
Austin Athman

Funding
The intramural program of NIAID, NIH
Creutzfeldt-Jakob Disease Foundation

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease

Acknowledgments

The Michael H. Cole Memorial Research Grant, contributed by Jeanne Cole

The Daniel L. Dolgin Celebration Grant, contributed by His Family

The Harvey L. Hall Memorial Grant, contributed by Lavonne C. Hall

The Katie Pohl Dopirak Memorial Research Grant, contributed by the Pohl and Dopirak Families

The Marsha K. Snively Memorial Research Grant, contributed by the Snively Family