Project Title:

"RT-QuIC assay on olfactory brushings in asymptomatic carriers of E200K PRNP mutation: an explorative study for establishing when a preventative therapy should be started "

Gianluigi Zanusso

Department of Neurosciences, Biomedicine and Movement Sciences

University of Verona

Genetic Prion Diseases

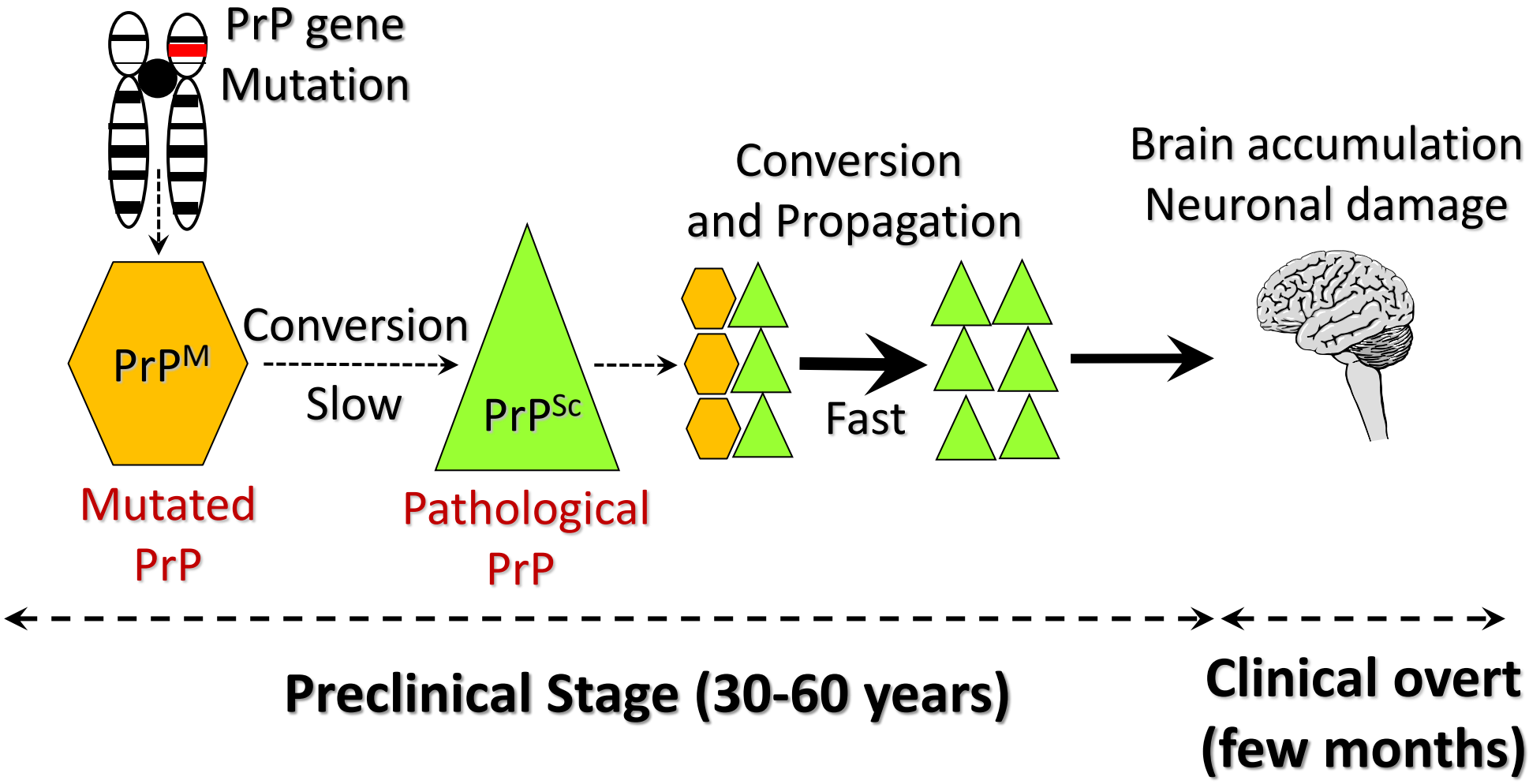
- Human PrP gene (*PRNP*) mutations correlate to distinct disease phenotypes such as: Genetic Creutzfeldt-Jakob Disease (gCJD)
  Fatal Familial Insomnia
  Gerstmann Straussler Scheincker syndrome
  PrP systemic amyloidosis

- The disease phenotype of gCJD is characterized by a rapidly progressive neurological disorder

- Mutation carriers of E200K develop a gCJD. E200K is diffuse worldwide with clusters in Slovakia, Italy (Calabria region), and among Libyan Jews in Israel

- For unknown reasons, the penetrance of E200K mutation varies among clusters and the probability to develop CJD in Calabrians is 50% at the age of 60 and 61% at the age of 70 *(D' Alessandro M. et al. Lancet 1998)*

Prion Conversion and Replication in Genetic Prion Diseases

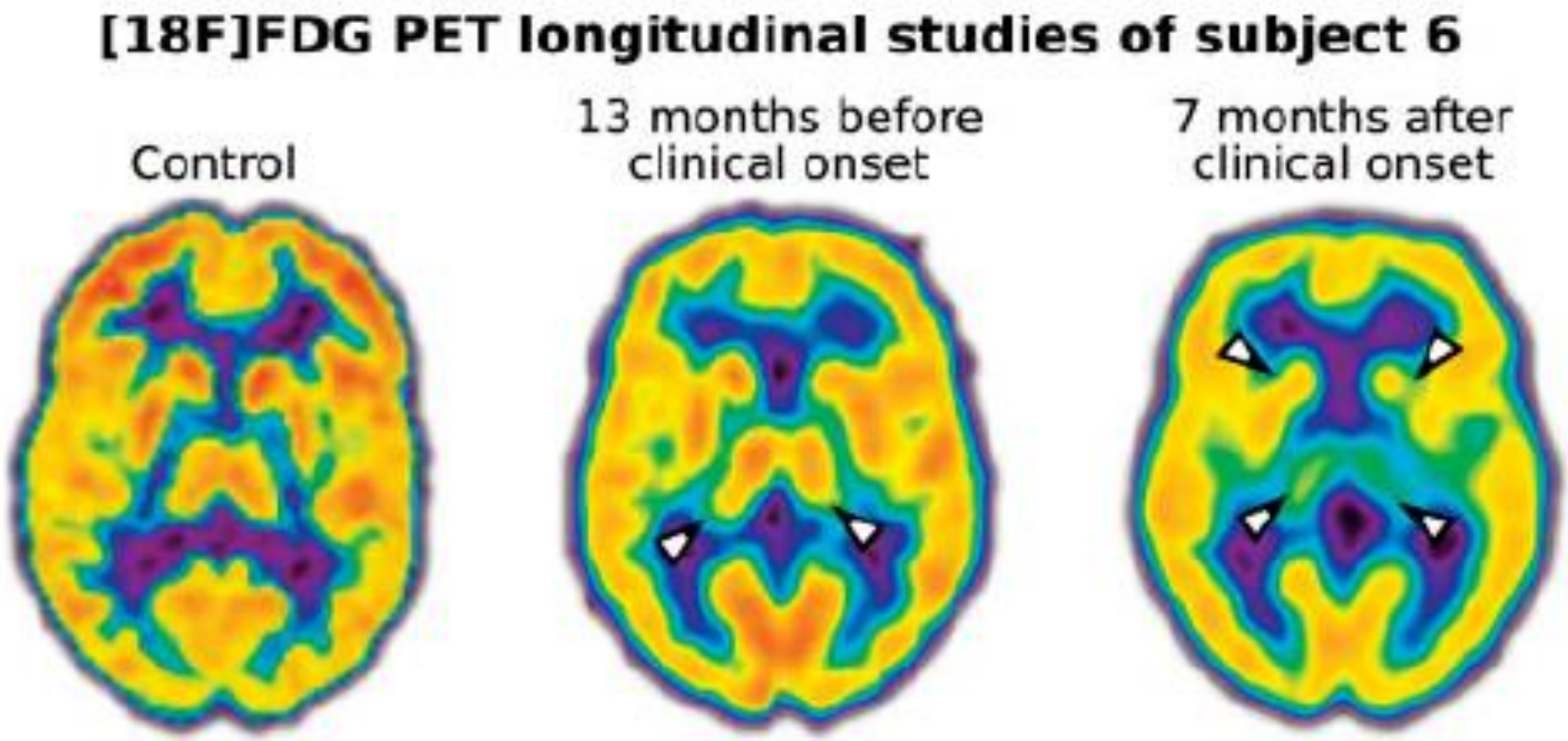


Pre-clinical Diagnosis of Prion Disease ?

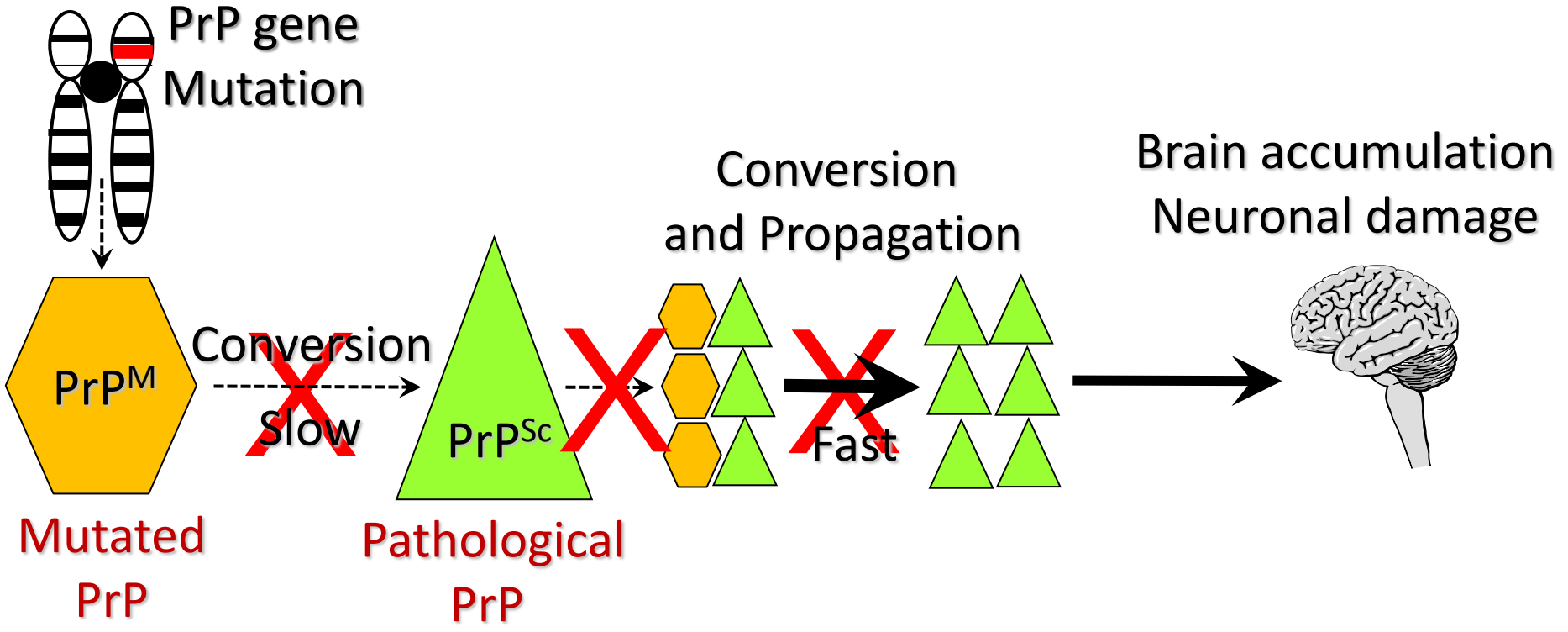
doi:10.1093/brain/awl003 *Brain* (2006), **129**, 668–675

Pre-symptomatic diagnosis in fatal familial insomnia: serial neurophysiological and ^{18}FDG-PET studies

Pietro Cortelli,[3] Daniela Perani,[1] Pasquale Montagna,[3] Roberto Gallassi,[3] Paolo Tinuper,[3] Provini Federica,[3] Patrizia Avoni,[3] Franco Ferrillo,[4] Davide Anchisi,[1] Rosa Maria Moresco,[2] Ferruccio Fazio,[2] Piero Parchi,[3] Agostino Baruzzi,[3] Elio Lugaresi[3] and Pierluigi Gambetti[5]



Prion Conversion and replication in Genetic Prion Diseases

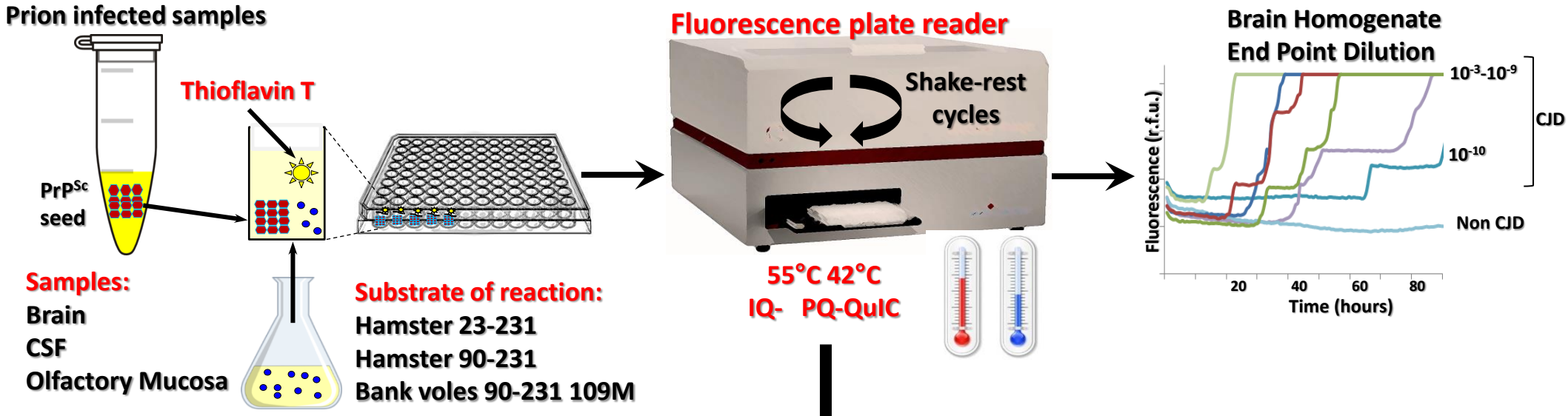


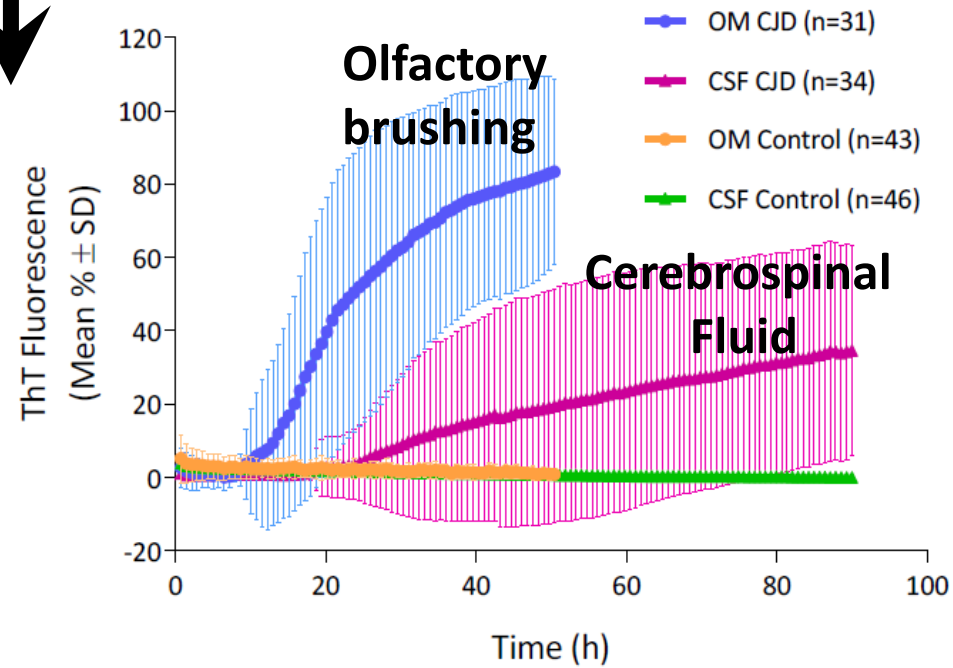
Preclinical Stage (30-60 years)

Clinical overt (few months)

Potential therapetutic window

RT-QuIC Assay and Olfactory Brushing





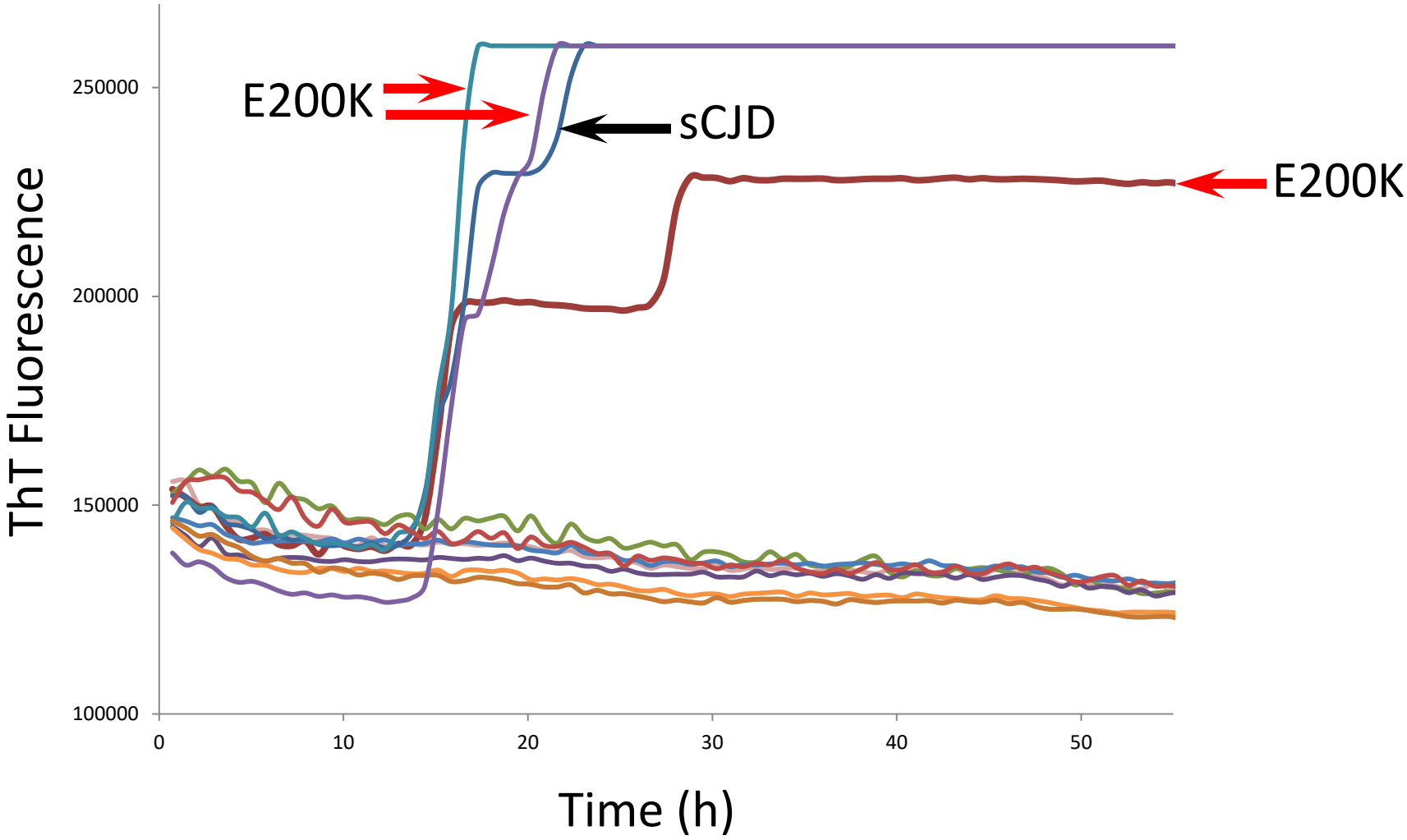
Short movie of Nasal Brushing Procedure

Diagnostic Accuracy of OM brushing coupled with RT-QuIC

Olfactory Mucosa brushing is:

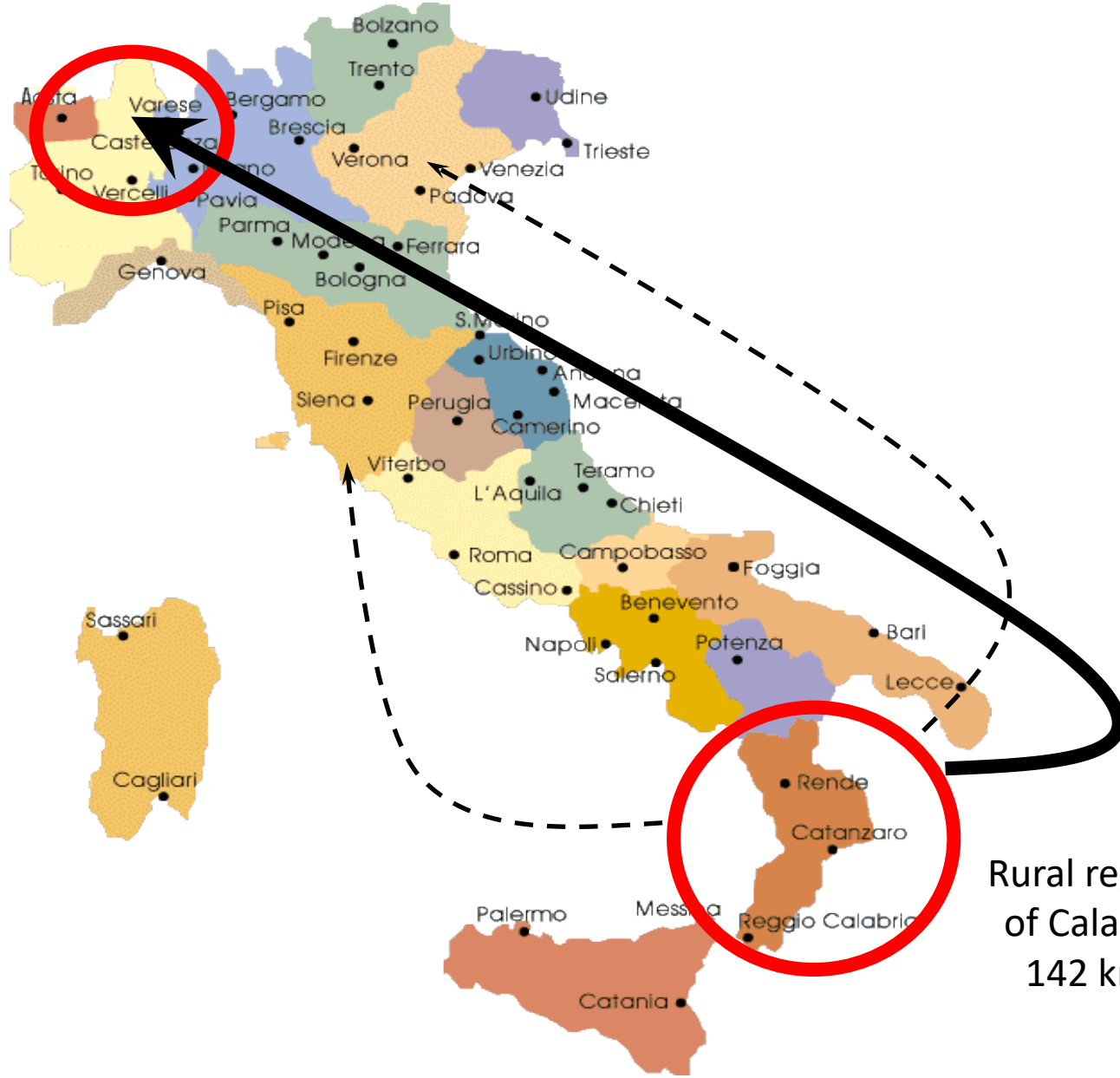
- **Easily performed:** It does not require trained otolaryngologists
- **Non-invasive:** No medical or surgical complications or a smell disorder
- **Highly efficient:** It collects around 300.000 olfactory neurons with a single sampling
- **Highly sensitive:** RT-QuIC assay of OM detects pathological PrP seeding activity in OM of CJD patients with a 97% sensitivity and 100% specificity

RT-QuIC assay in Olfactory Brushingsof patients with sporadic CJD and E200K using Hamster PrP 23-231 at 42°C



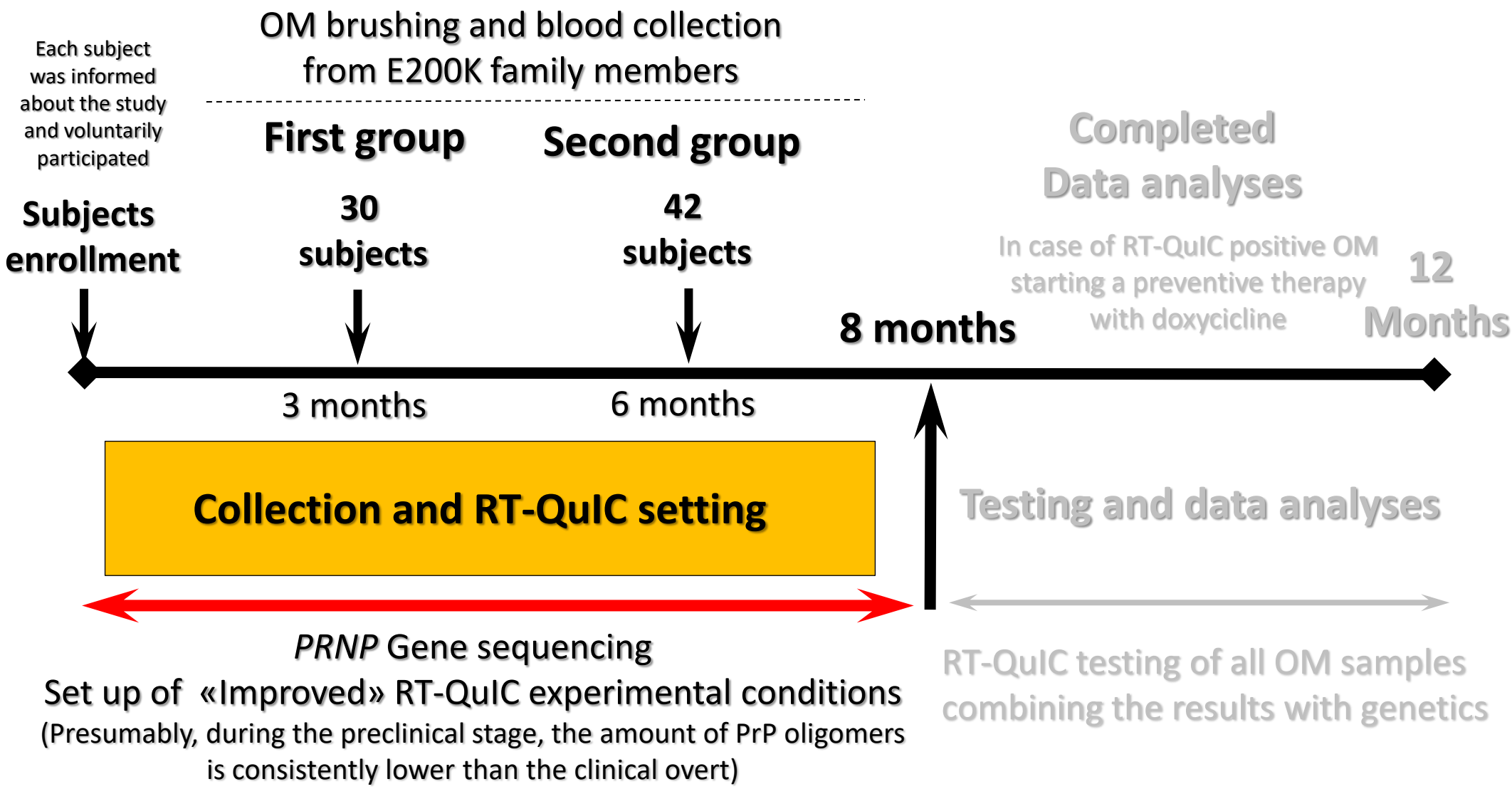
Migration of E200K family members from rural region of Calabria to the Northern Italy

E200K cluster where the preclinical OM testing was setting up

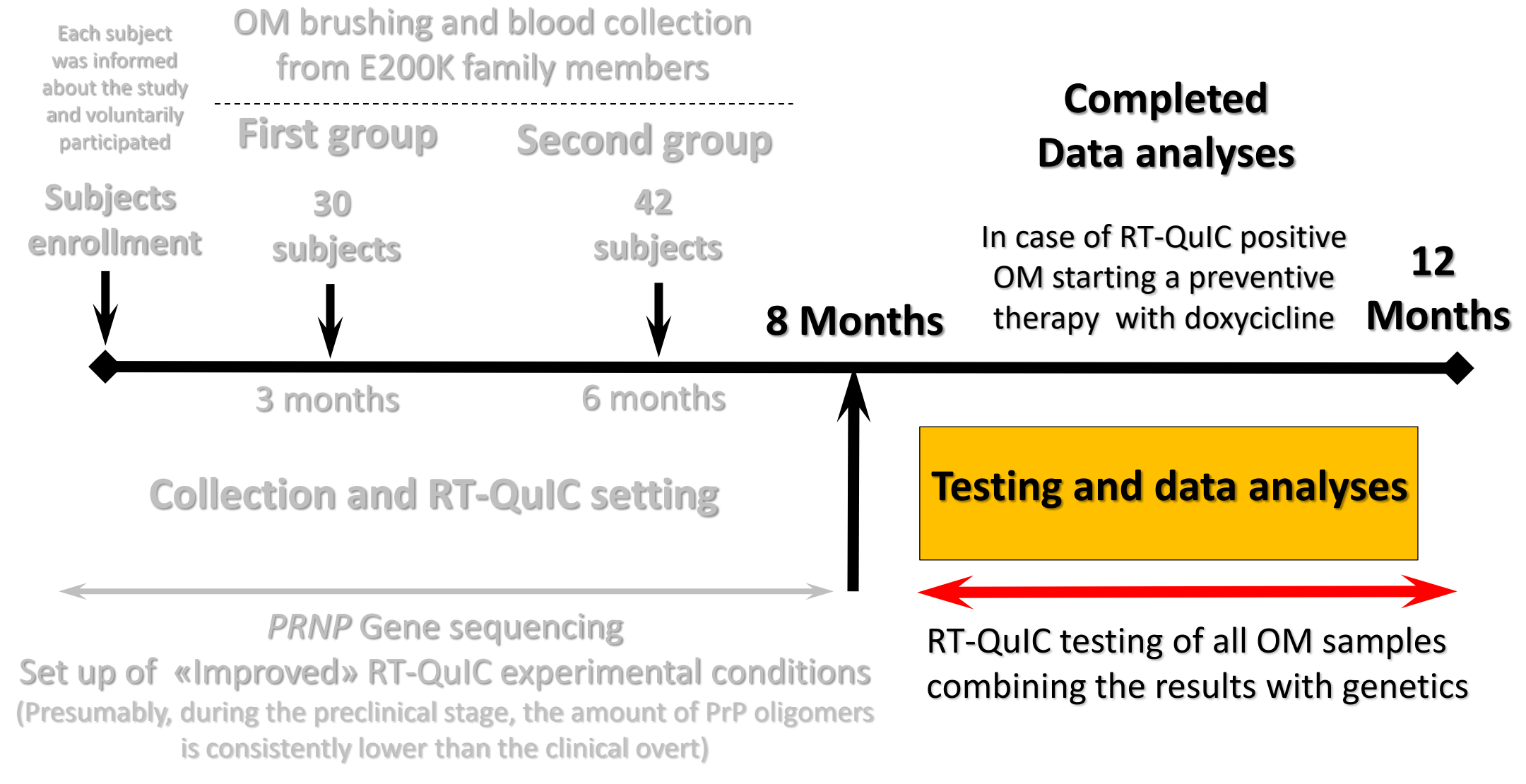


Rural region of Calabria 142 km^2

Study Plan: First Part



Study Plan: Second Part

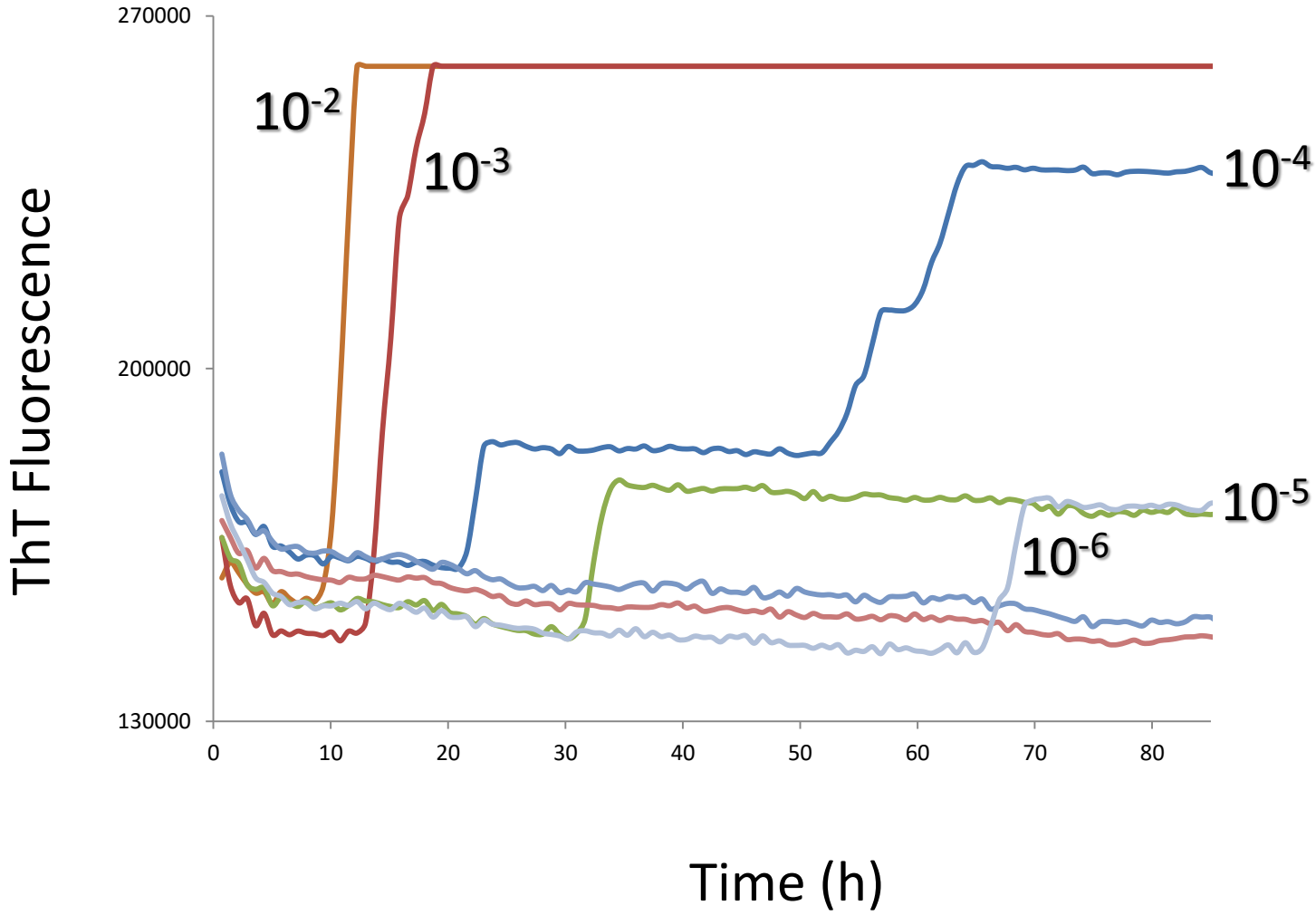


State of Art

- 72 subjects enrolled: 30 Male and 42 Female
- Mean age: 51.2 (range 19-90)
- All undewent to OM brushing (except two) and blood withdrawal
- No complications were observed and all OM samples were suitable for the test
- Genetic analysis: 21 carriers of E200K mutation

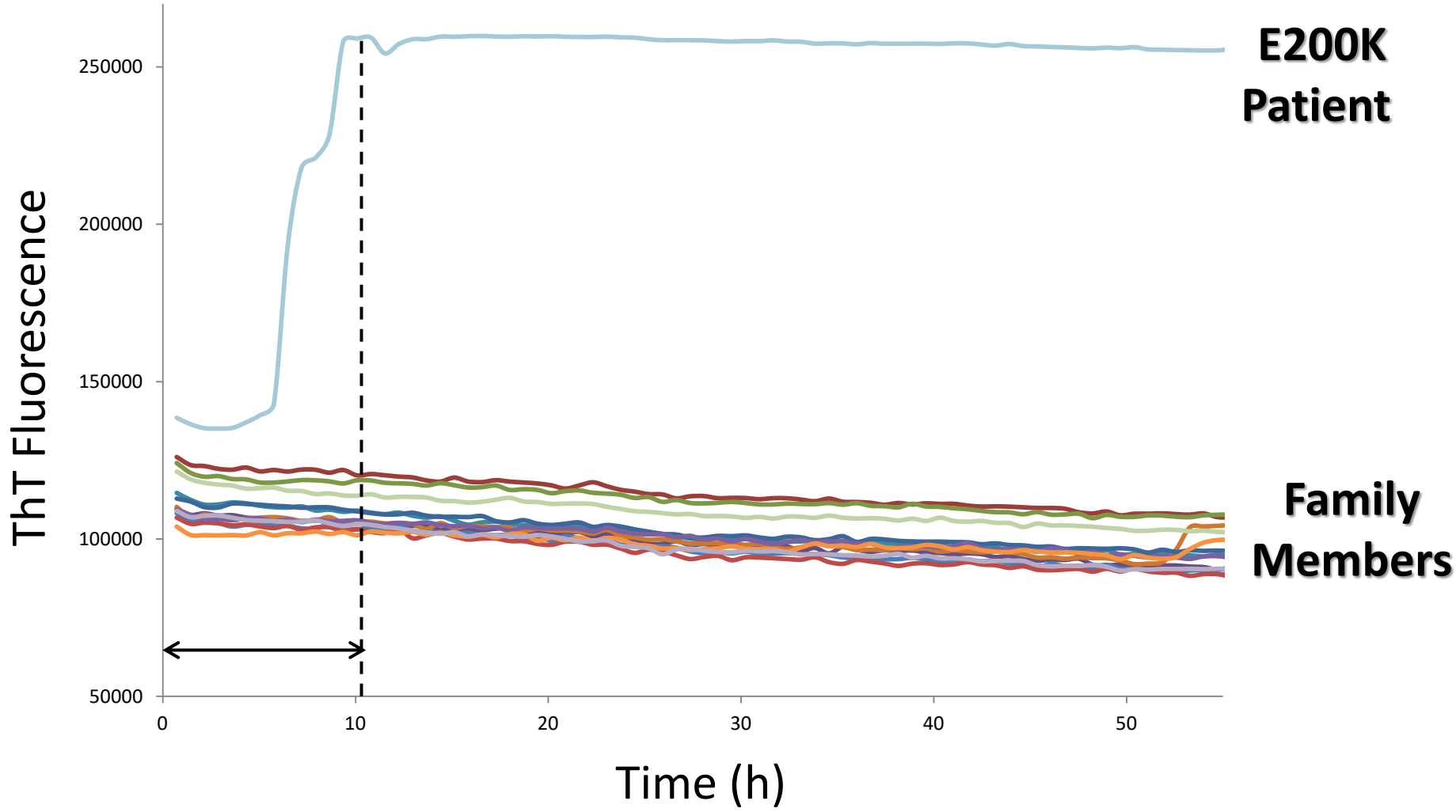
End Point Dilution of OM from patients with E200K *PRNP* mutation

(Hamster PrP 23-231 at 42°C)



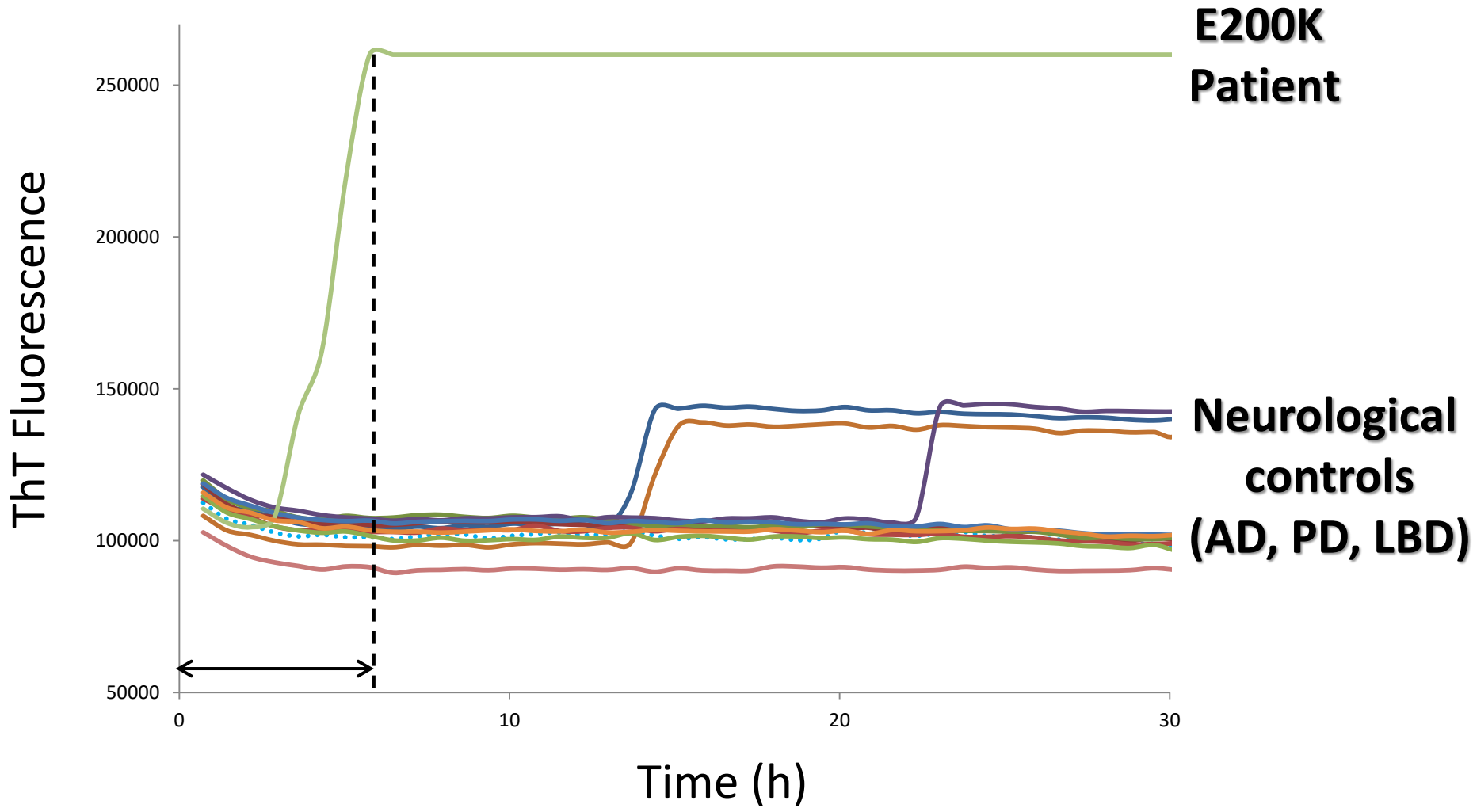
RT-QuIC testing of OM Using Standard Conditions

(Hamster PrP 23-231 at 42°C)



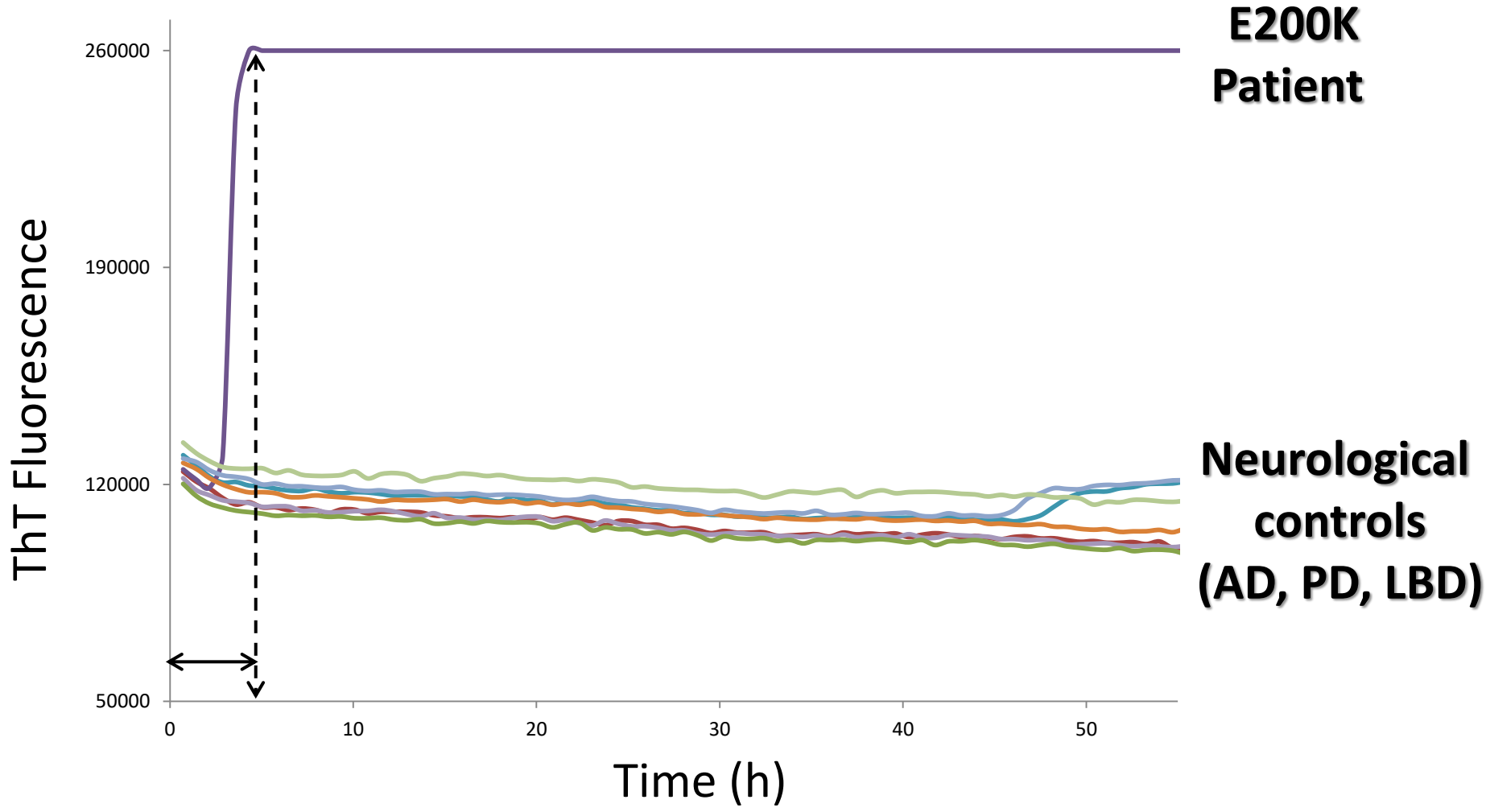
RT-QuIC testing of OM using «Improved» RT-QuIC Conditions

(Hamster PrP 90-231 at 55°C)



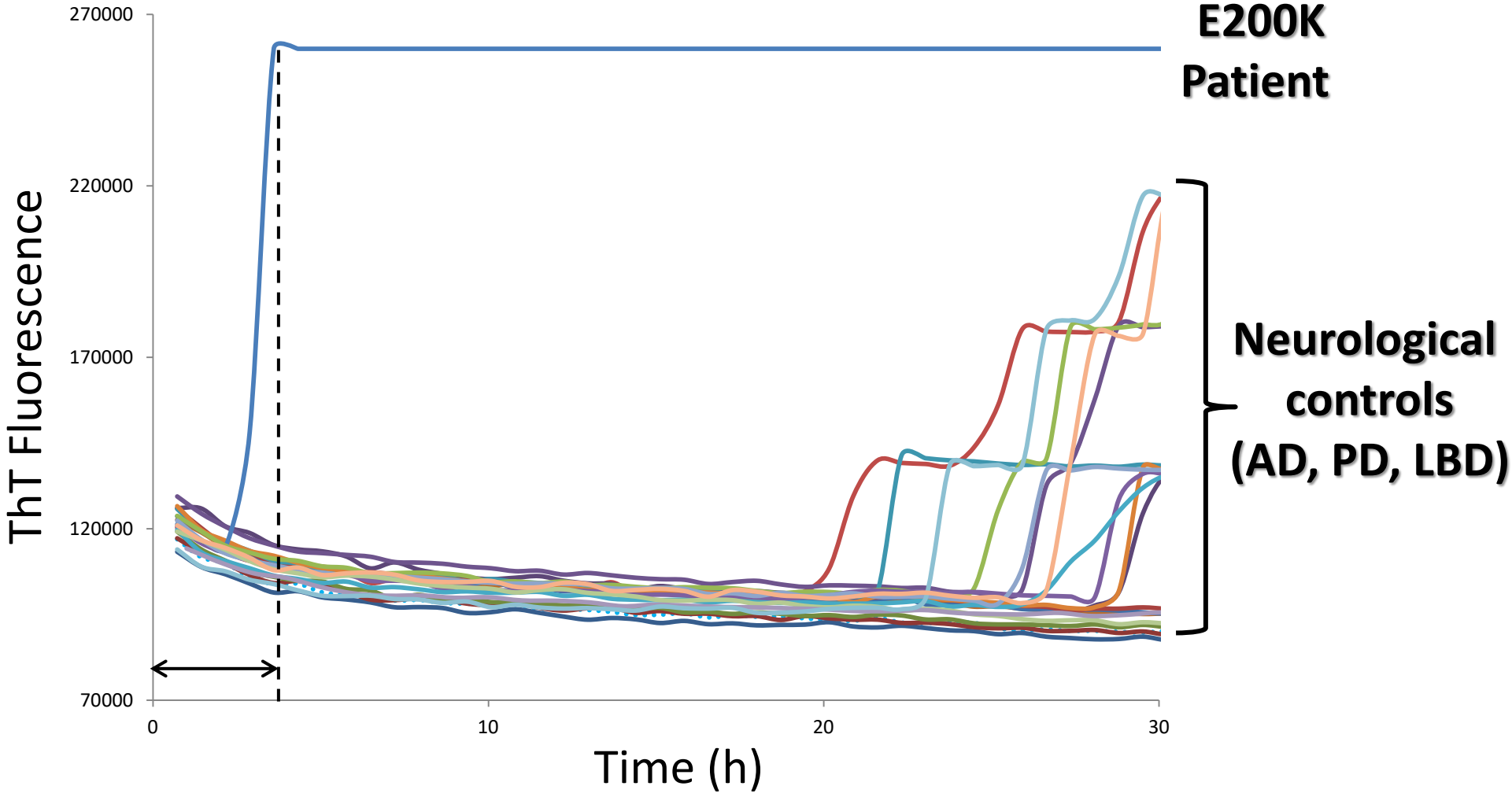
RT-QuIC testing of OM using «Improved» RT-QuIC Conditions

(Hamster PrP 23-231 at 55°C)



RT-QuIC testing of OM using «Improved» RT-QuIC Conditions

(Vole PrP 90-231 109M at 55°C)



Summary

- Nasal brushing procedure was well tolerated by all subjects;
- The end-point dilution of OM from patients with E200K, using RT-QuIC standard conditions (Hamster PrP 23-231 at 42°C), was 10^{-6}. No positive sample in OM from healthy family members;
- We tested different «Improved» RT-QuIC experimental conditions, changing PrP substrates of reaction and temperatures, for increasing the sensitivity without compromising the specificity of the test;
- We determined that the most appropriate «Improved condition» was Hamster PrP 23-231 at 55°C (OM resulted positive after 5 hours in E200K patient without false positive in other neurodegenerative disorders).