ROBERT KOCH INSTITUT

Decontamination of Medical Devices from Human Prions

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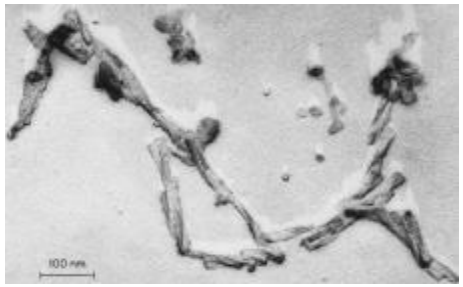
Michael Beekes, Marc Thanheiser, Katja Wagenführ

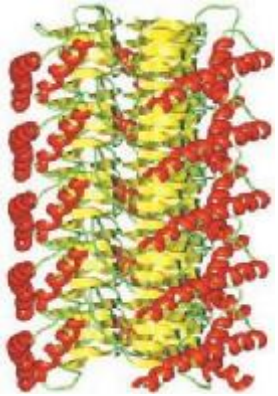
An Unconventional Biological Principle of Infection

Prions

- Proteinaceous infectious particles.
- Cause and transmit fatal neurodegenerative brain diseases.
- Consist essentially of misfolded, aggregated prion protein (PrP^{TSE}) derived from a host-encoded cellular precursor (PrP^{C}).

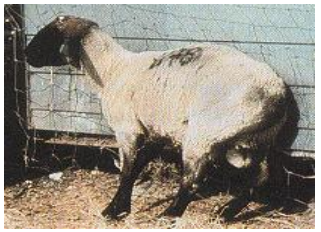


M. Özel & H. Diringer, Robert Koch-Institut



Govaerts et al., 2004,
Proc Natl Acad Sci USA,
101: 8342-8347

Prion Diseases of Animals and Humans

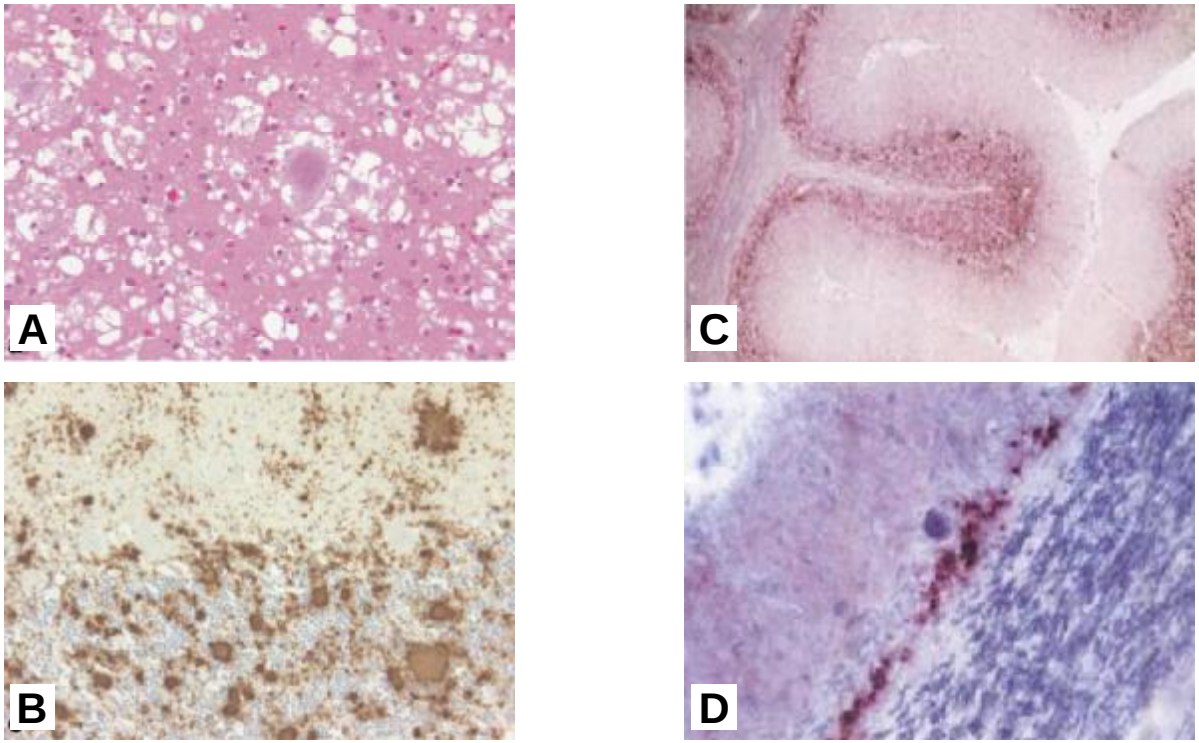


- **Scrapie**
  Sheep and goat
- **Bovine Spongiform Encephalopathy (BSE)**
  Cattle

- **Chronic Wasting Disease (CWD)**
  Deer and elk
- **Creutzfeldt-Jakob Disease (CJD)**
  Human
- **Variant Creutzfeldt-Jakob Disease (vCJD)**
  Human

Cerebral Deposition of Pathological Prion Protein in Patients with Creutzfeldt-Jakob Disease

Variant CJD (vCJD) | **Sporadic CJD (sCJD)**

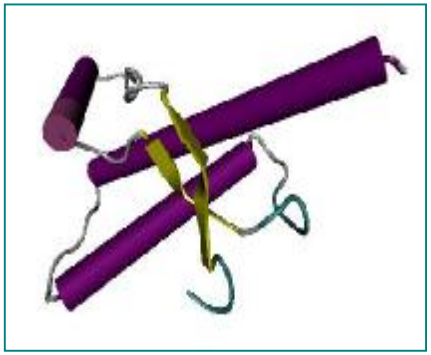


Pathological prion protein deposits in the brain of patients with vCJD (A, B) or sporadic CJD of type MM1 (C) or VV2 (D). A: haematoxylin-eosin staining; B-D: anti-PrP immunostaining.

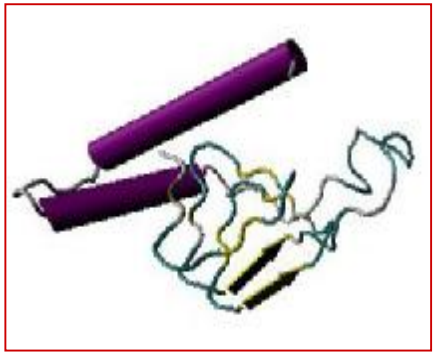
A, B: Ironside et al., 2002, APMIS, 110: 79-87; C, D: Kretzschmar & Parchi, 2006. In: Prions in Animals and Humans: 287-314

Conformational Transition of Normal into Pathological Prion Protein

PrP^{C}



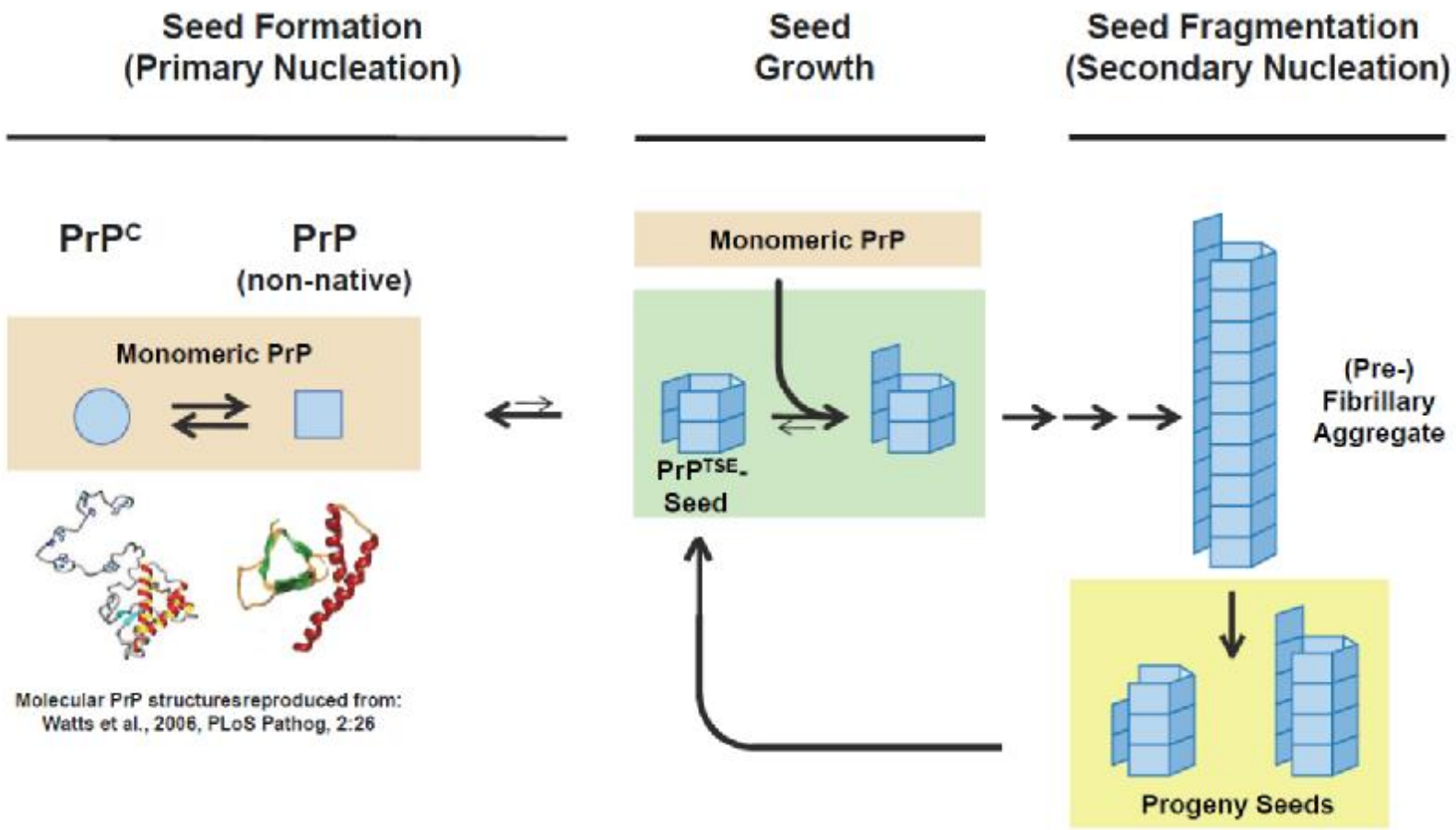
PrP^{Sc}/PrP^{TSE}



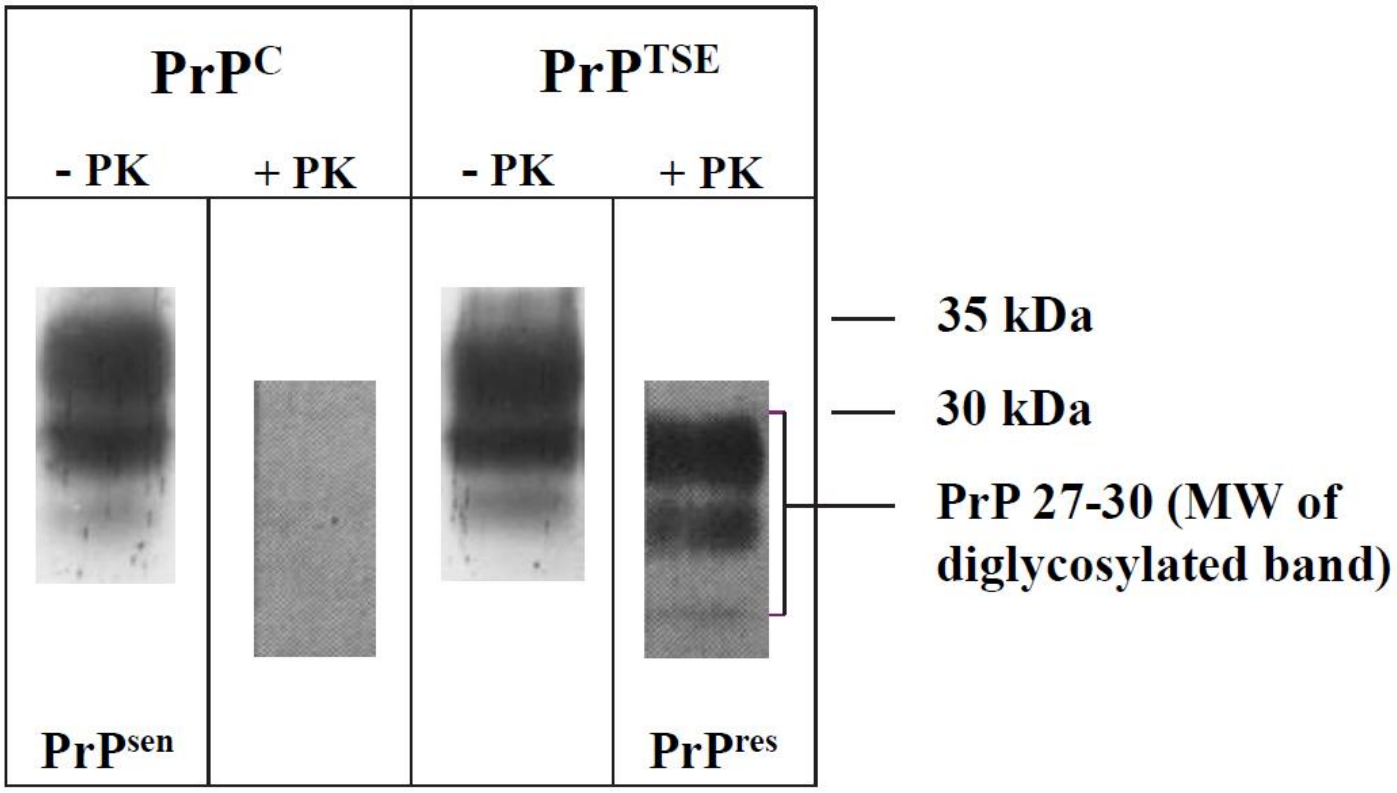
- Misfolded
- Aggregated
- Protease-resistant
- Associated with infectivity

Þ **Neurodegeneration**

Formation and Replication of Prions:
The Nucleation-Dependent Polymerization Model



Detection and Discrimination of Normal and Aberrant Prion Protein by Western Blotting



Western blots of PrP from hamsters (mAb 3F4)

Þ **PrPres: Frequently used as surrogate marker for prions.**

Tolerance of Prions to Disinfection Procedures

No detectable infectivity	Significant titre reduction	Little titre reduction
Sodium hypochlorite (16,500 ppm available chlorine)	1 *M* or 2 *M* sodium hydroxide	aldehydes
Autoclaving at 121 °C after 1 *M* sodium hydroxide treatment	sodium dichoroisocyanurate (16,500 ppm available chlorine)	organic solvents phenolic disinfectants chlorine dioxide iodine and iodates
Autoclaving at 121 °C in 1 *M* sodium hydroxide	chaotropes (e.g. guanidine thiocyanate)	peracetic acid protcolytic enzymes microwave irradiation
Boiling in 1 *M* sodium hydroxide	95% formic acid hot 1 *M* hydrochloric acid autoclaving for 18 min at 134–138 °C autoclaving for 1 h at 132 °C autoclaving at 121 °C in 5% sodium dodecyl sulphate dry heat at >200 °C	UV irradiation gamma irradiation autoclaving after aldehyde, alcohol or dry heat treatments

Reproduced with modifications from: Taylor, 2004, Contrib Microb, 11: 136-145

Hierarchy of Microbial Tolerance to Disinfection

Micro-organism	Examples
Prions	Scrapie, Creutzfeld–Jakob disease, chronic wasting disease
Bacterial spores	*Bacillus, Geobacillus, Clostridium*
Protozoal oocysts	*Cryptosporidium*
Helminth eggs	*Ascaris, Enterobius*
Mycobacteria	*Mycobacterium tuberculosis, M. terrae, M. chelonae*
Small, non-enveloped viruses	Poliovirus, parvoviruses, papilloma viruses
Protozoal cysts	*Giardia, Acanthamoeba*
Fungal spores	*Aspergillus, Penicillium*
Gram-negative bacteria	*Pseudomonas, Providencia, Escherichia*
Vegetative fungi and algae	*Aspergillus, Trichophyton, Candida, Chlamydomonas*
Vegetative helminths and protozoa	*Ascaris, Cryptosporidium, Giardia*
Large, non-enveloped viruses	Adenoviruses, rotaviruses
Gram-positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*
Enveloped viruses	Human immunodeficiency virus, hepatitis B virus, herpes simplex virus

McDonnell & Burke, 2011, J Hosp Infect, 78: 163-170

Prions – Challenging Agents for the Reprocessing of Medical Devices

CJD

Incidence of sporadic CJD:

1-2 cases per 1 million persons per year.

Prions are essentially confined to the CNS.

Table 1 Total cases of iatrogenic CJD world-wide

Mode	Cases (*n*)	Mean incubation period (years)	Clinical
Neurosurgery	4	1.6	Visual/cerebellar/dementia
Depth electrodes	2	1.5	Dementia
Corneal transplant	3	15.5[a]	Dementia
Dura mater	136	6[b]	Visual/cerebellar/dementia
Human growth hormone	162	12[b]	Cerebellar
Human gonadotrophin	5	13	Cerebellar

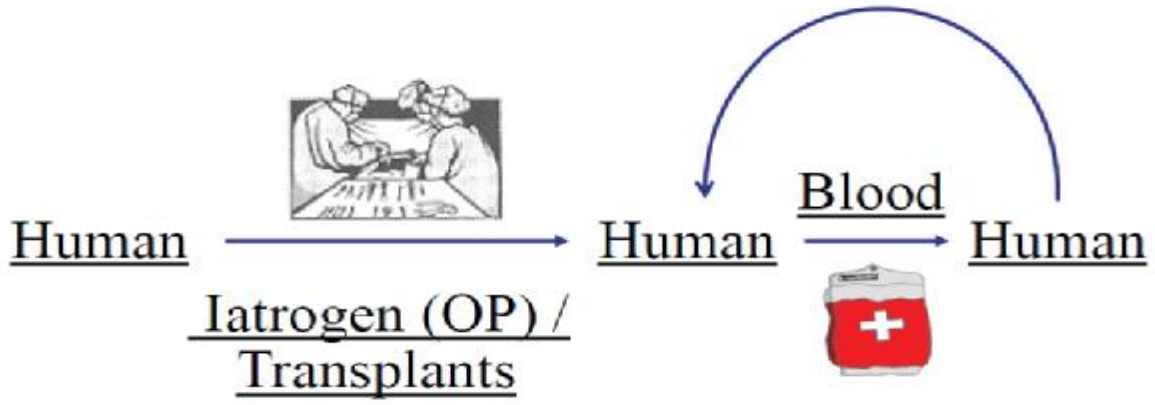
Will, 2003, Brit Med Bull, 66: 255-265

vCJD

Prevalence of asymptomatic vCJD infections within the UK population:

One person in 2000 in the 1941-85 birth cohort.

Prions are present in the CNS and in peripheral tissues.



Iatrogenic Risks of CJD/vCJD Prompted Detailed Guidelines for Prion-Effective Reprocessing of Medical Devices

Germany

- **Recommendations of Task Force vCJD**

The routine reprocessing of medical devices not used on "risk patients" should include a minimum of two procedures that are at least partially effective against prions.

How to Test Cleaners, Disinfectants and Heat Sterilization for Efficacy Against Prions?

n Tube Assay

Contamination with prion brain homogenate

Drying

Chemical / thermal treatments

Analysis of prion depletion

n Carrier Assay

Contamination with prion brain homogenate

Chemical / thermal treatments

Analysis of prion depletion

Adapted from Zobeley et al. (1999, Mol Med, 5, 240-243) and Flechsig et al. (2001, Mol Med 7, 679-684)

Analytical Methods for the Assessment of Prion Depletion in Tube- and Carrier Assays

- Test the depletion of PrPres.
- Test the reduction of prion seeding activity in PrP^{C} ® PrPres conversion assays.
- Test the reduction of prion infectivity in cell assays.

In vitro-assays

- Validate the reduction of prion infectivity in animals.

In vivo-assays

Well established for rodent prions but not always similarly feasible with human prions.

Efficacy Testing of Cleaners, Disinfectants and Heat Sterilization in Assays with Rodent-Adapted Prions

- Common practice for many years.
- Based on continuously improved and often widely-used test systems.
- Allows relatively high throughput .
- Thought to provide a reliable model for the removal / inactivation of human prions.

But.....

Reports on a Higher Tolerance of Human than Rodent Prions to Chemical or Thermal Treatments

- Hamster scrapie prions may be 100 000 times less resistant to inactivation by acidic SDS than prions from patients with sporadic CJD.[1]

- Prions of variant CJD appear to be more resistant to steam sterilization than prions that are not derived from BSE (e. g. rodent scrapie agents).[2]

- **Efficacy of chemical or thermal treatments against model prions cannot be generally extrapolated to and needs to be validated for sCJD and vCJD agents.**

[1]Peretz et al., 2006, J Virol, 80: 322-331; [2]Fernie et al., 2012, J Hosp Inf, 80: 46-51

Methodological Difficulties of Validation Studies with Human Prions

- Limited availability of brain tissue from human sCJD and vCJD patients for decontamination / inactivation studies.
- Existence of different sCJD forms possibly associated with distinct prion strains.
- Lack of commonly available rodent models or cell assays that allow the titration of sCJD and vCJD agents with similarly high sensitivity as possible for hamster or mouse prions.

Rationale for Pilot Validation Studies with Human Prions

Test prions

- vCJD, sCJD/MM1, sCJD/VV2

In vitro tests

- PrPres depletion assay
- Biochemical PrP^{C} ® PrPres conversion assay measuring prion-associated seeding activity

Test treatments

- Cleaner / disinfectant formulations
- Heat Sterilization (134 °C, different holding times)

Is Broad-Range Disinfection of Bacteria, Viruses, Fungi and Prions Feasible for vCJD or sCJD agents?

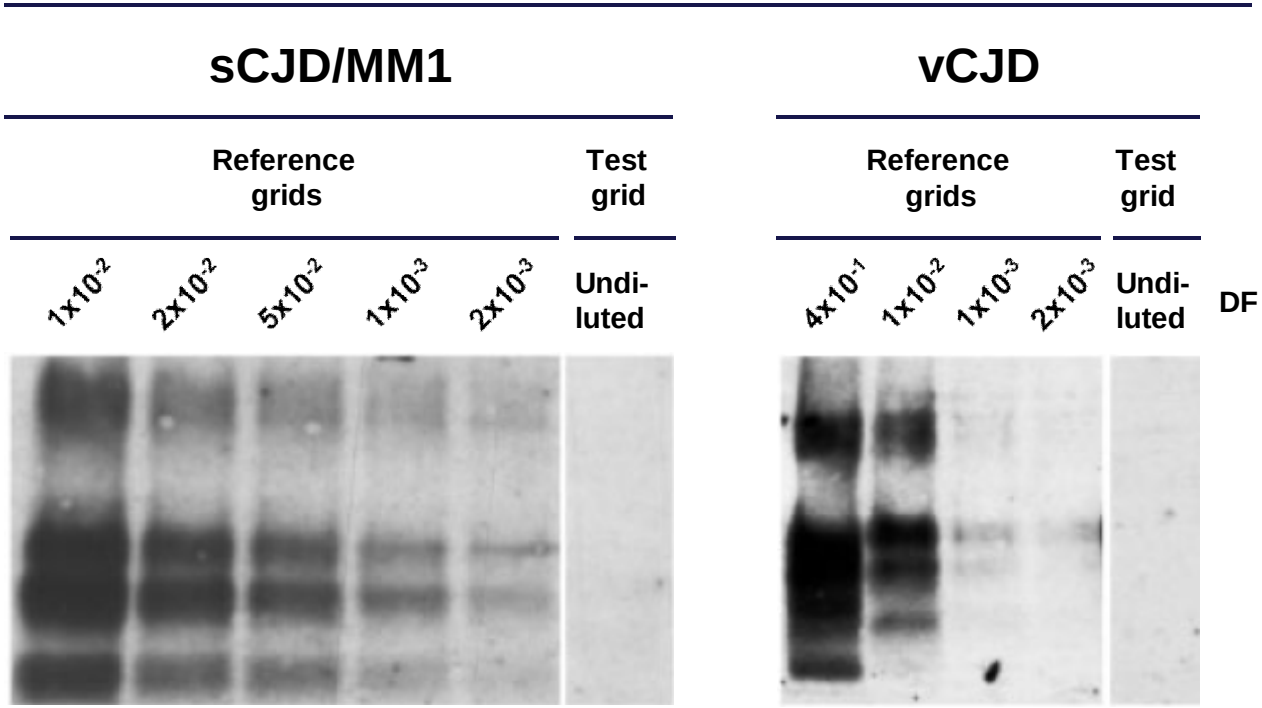
Titre reductions with
0.2% SDS / 0.3% NaOH / 20% n-Propanol (20 min, RT, pH 13)

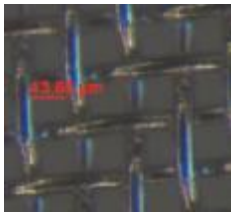
■ **Prions** (263K Scrapie – Agent on Steel Wires)	≥ 5 logs
■ **Bakteria / Mycobacteria** (*E. faecium, M. avium*)	≥ 6 logs
■ **Viruses** (Poliovirus and Hepatitis A – Virus on Steel Wires with Coagulated Blood)	≥ 4 logs
■ **Fungi** (Spores of *Aspergillus niger*)	≥ 5 logs

Beekes et al. (2010), J Gen Virol, 91: 580-589

Chemical Depletion of sCJD/MM1 and vCJD-Associated PrPres on Test Carriers

Formulation: ***0.2% SDS / 0.3% NaOH / 20% n-Propanol (20 min, RT)***





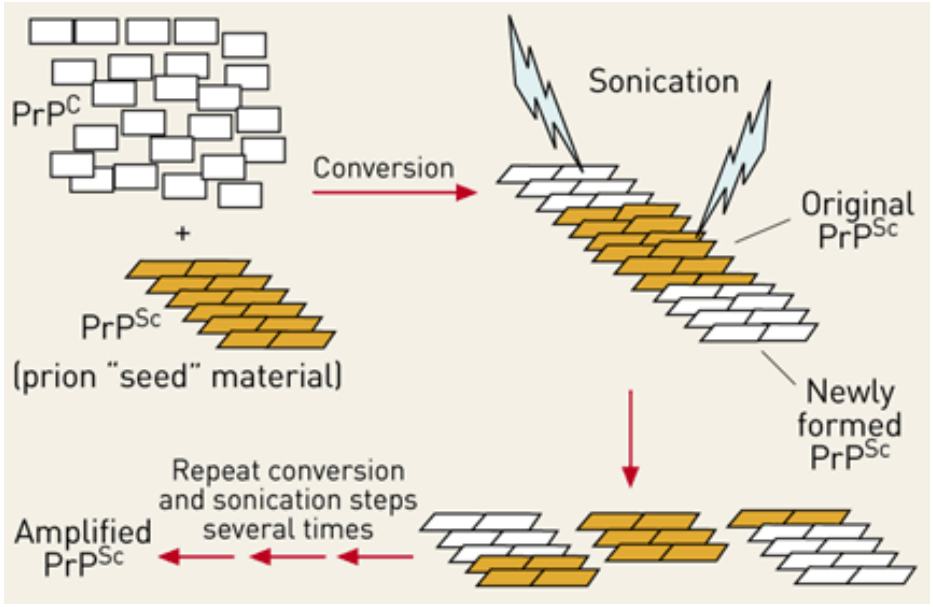
100 x 5 mm

Depletion of PrP^{res}: ~ 3.3 logs (test limit)

Eluates from reference steel wire grids contaminated with 25% sCJD/MM1- or vCJD brain homogenate, and from reprocessed test steel wire grids originally contaminated with identically concentrated brain homogenates. DF: Dilution factor.

Beekes et al., 2010, J Gen Virol, 91: 580-589

Cell-Free Detection of Prion Seeding Activity by Protein Misfolding Cyclic Amplification (PMCA)



Saborio et al., 2001, Nature, 411: 810-813

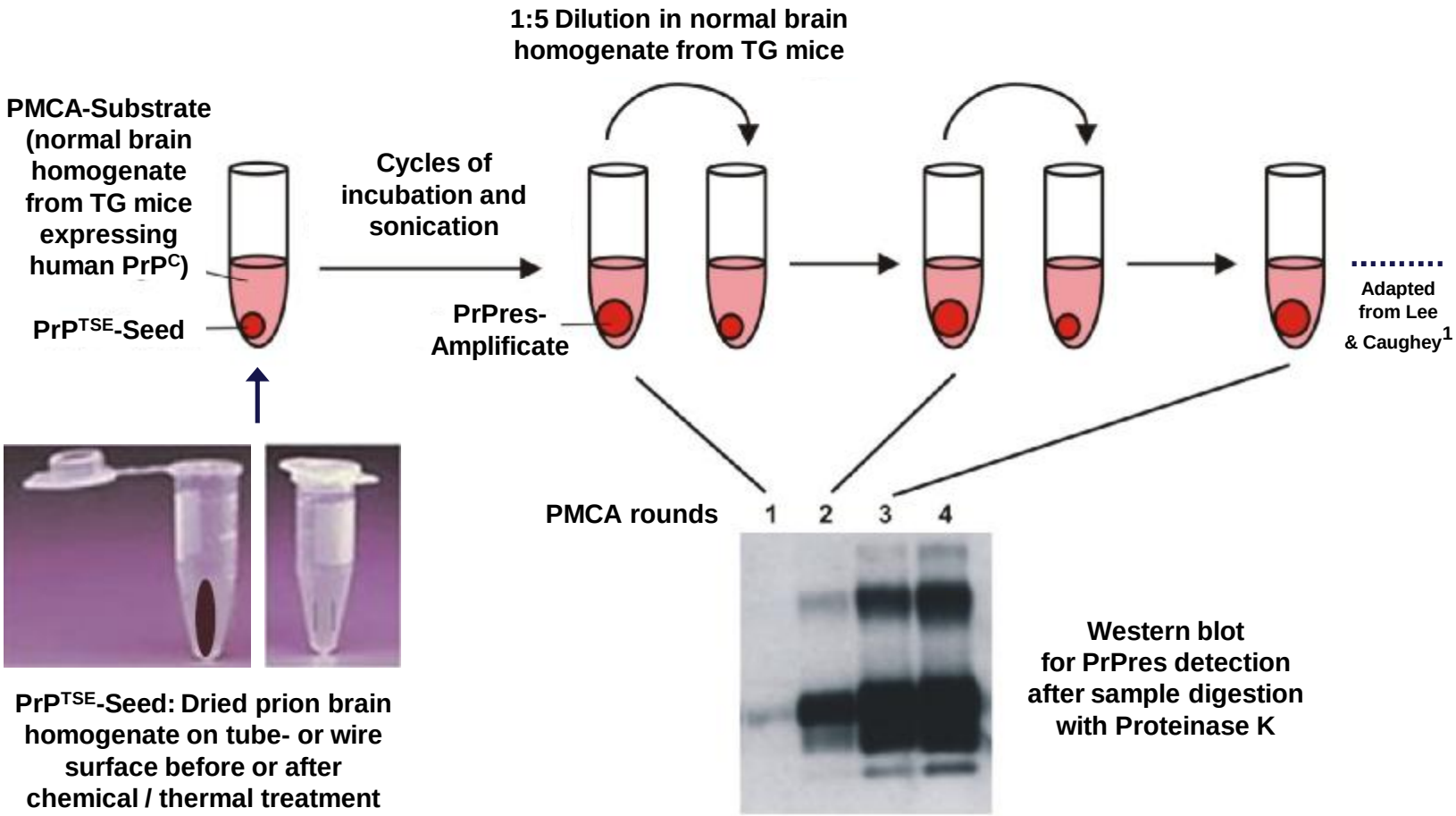
PMCA

brain homogenate
+
infectious seed

sonication

incubation

Quantitative PMCA for Seeding Activity Testing in Tube- and Carrier Assays



[1]Lee & Caughey, 2007, Proc Natl Acad Sci USA, 104: 9551-9552

Sensitivity of PMCA: A Hypothetical Illustration

Detection limit for seeding activity of 263K scrapie prions:

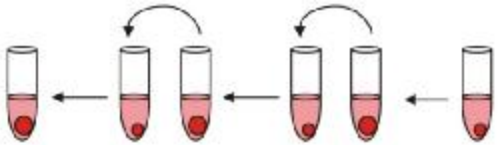
1x10^{-12} g brain tissue

Brain of a clinically diseased scrapie hamster (~1g)

Swimming pool: 50 m x 10 m x 2m

1 µl

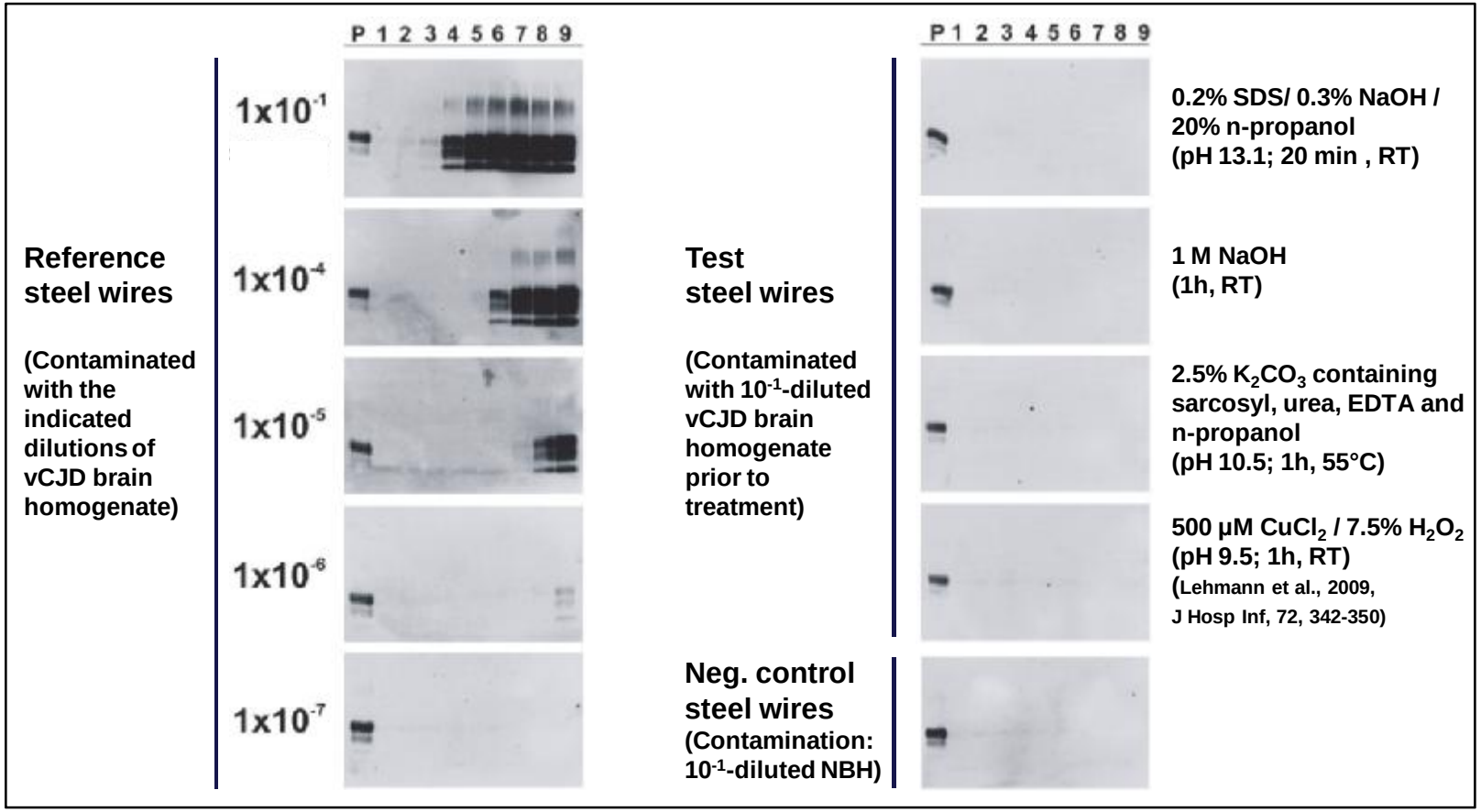
Analysis in PMCA assay



PMCA rounds

Chemical Reduction of vCJD-Associated Prion Seeding Activity on Test Carriers

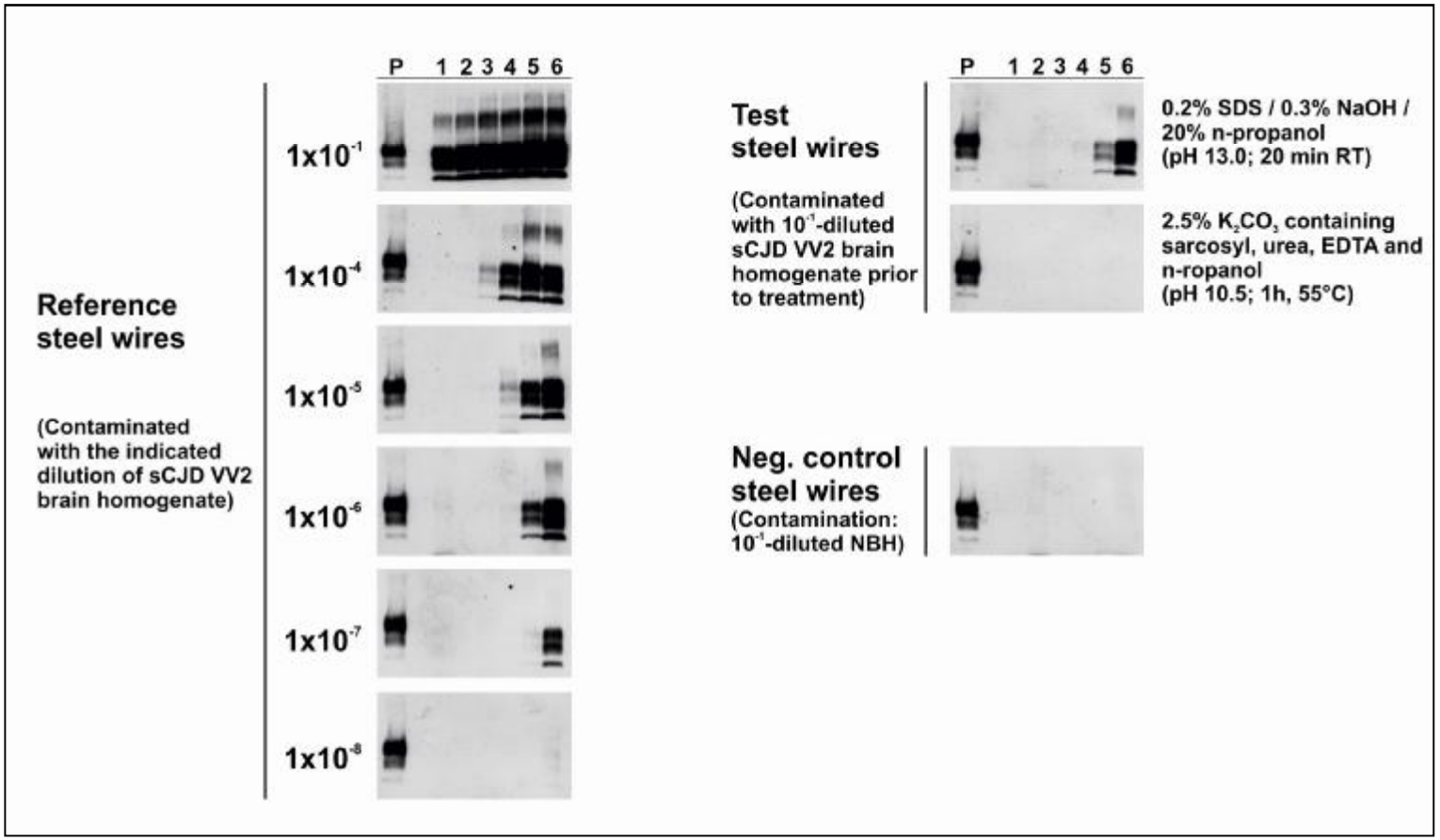
PMCA (9 rounds) with vCJD-contaminated steel wires



Þ **Reduction of seeding activity by the tested chemical treatments: > 5 logs**

Chemical Reduction of sCJD/VV2-Associated Prion Seeding Activity on Test Carriers

PMCA (6 rounds) with sCJD/VV2-contaminated steel wires



⇒ **Reduction of seeding activity by the tested chemical treatments:**
~ 5 logs (SDS/NaOH/n-propanol) and > 6 logs (K_2CO_3)

Summary (I)

Efficacy of chemical treatments against sCJD / vCJD prions

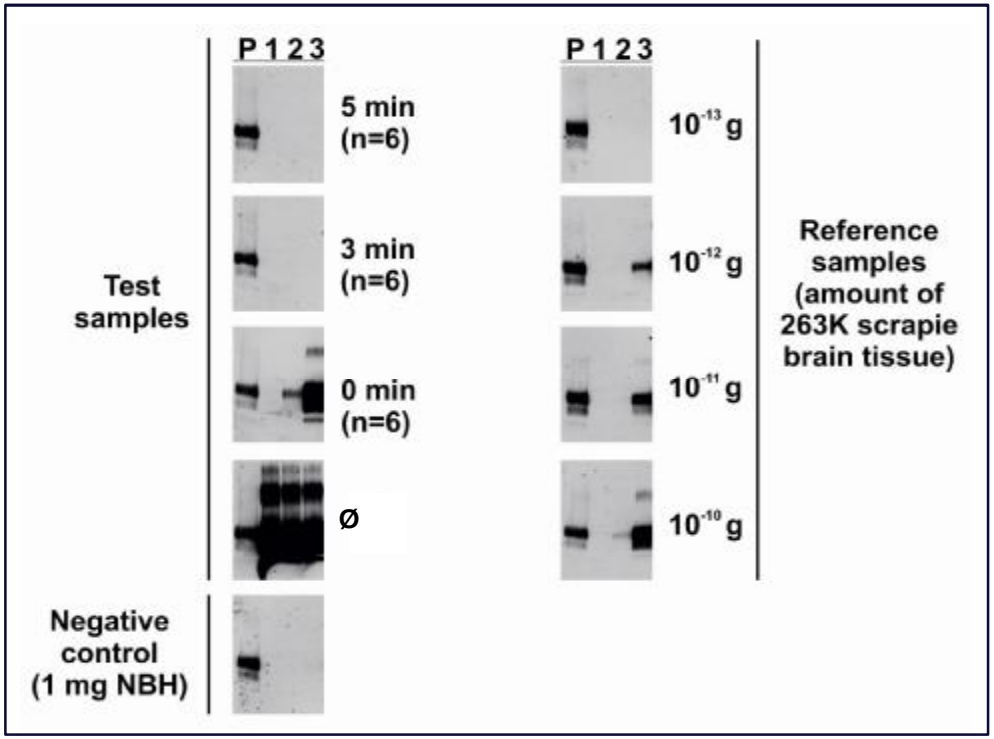
- **Depletion of PrPres on steel wire grids was tested with one formulation.**
- **Reduction of seeding activity on steel wires was tested with two/four formulations, respectively.**
- **Results were consistent with data from previous studies using 263K scrapie prions and suggest a high efficacy of the tested formulations against the examined human prions.**

Efficacy of Autoclaving Procedures Against Prion Seeding Activity: 263K Scrapie

- Autoclaving of 263K scrapie hamster brain homogenate (1 mg of tissue, 100 µg total protein)
- 134°C at holding times of 0, 3 or 5 min

- Reduction of seeding activity: > 9 logs

PMCA (3 rounds)

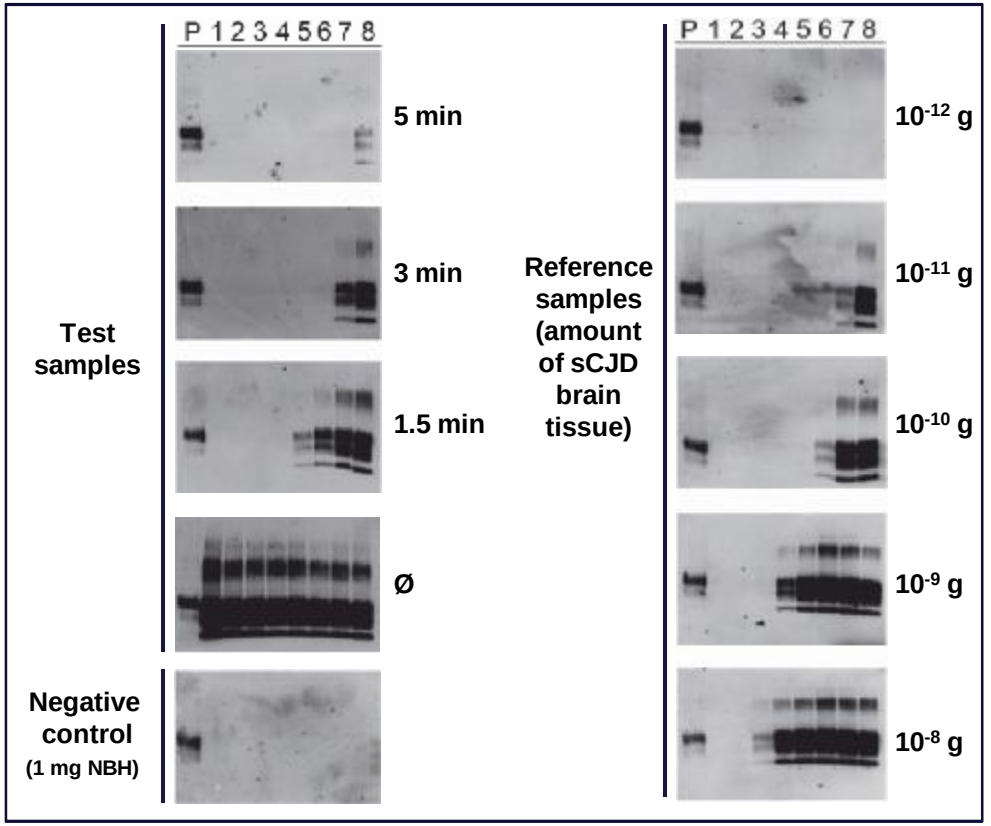


Efficacy of Autoclaving Procedures Against Prion Seeding Activity: Sporadic CJD/VV2

- Autoclaving of sCJD/VV2 brain homgenate (1 mg tissue, 100 µg total protein) at 134°C and holding times of 1.5, 3 and 5 min

- Reduction of seeding activity: > 8 to < 9 logs

PMCA (8 rounds)



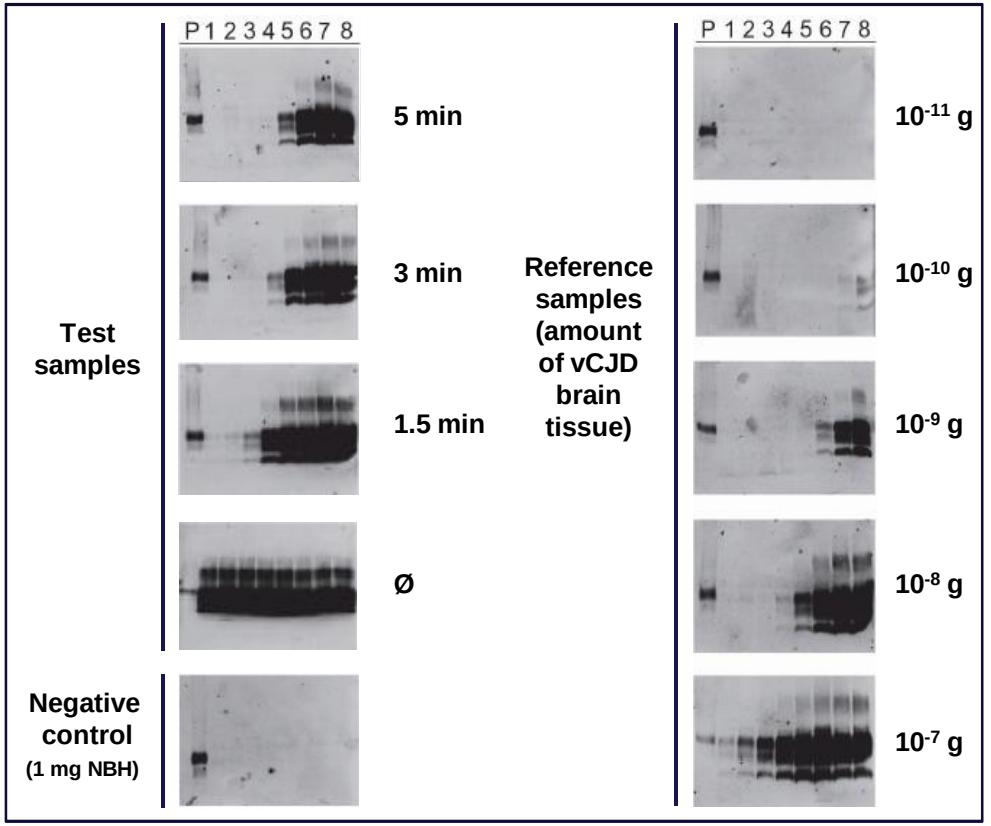
Preliminary data

Efficacy of Autoclaving Procedures Against Prion Seeding Activity: Variant CJD

- Autoclaving of vCJD brain homgenate (1 mg tissue, 100 µg total protein) at 134°C and holding times of 1.5, 3 and 5 min

- Reduction of seeding activity: > 5 to < 6 logs

PMCA (8 rounds)



Preliminary data

Summary (II)

Efficacy of heat sterilization against sCJD / vCJD prions

- **Autoclaving at 134°C for 5 minutes reduced the seeding activity of sCJD/VV2- and vCJD prions for > 8 to < 9 logs and > 5 to < 6 logs , respectively.**
- **Reduction of sCJD/VV2-associated seeding activity after autoclaving for 5 minutes was basically consistent with data from heat sterilization studies using the 263K scrapie agent.**
- **However, vCJD-associated seeding activity showed a substantially higher tolerance to autoclaving at 134°C than 263K scrapie prions.**

Outlook

- **Cleaners, disinfectants and heat sterilization processes routinely used for the reprocessing of medical devices should be systematically validated in terms of their efficacy against human prions.**
- **Such validations will become more feasible with increasing availability of robust cell-free and cell-based in vitro assays for human prions.**
- **The reliability and credibility of such in vitro assays can be strengthened by using complementary cell-free and cell-based approaches for monitoring the reduction of prion seeding activity and infectivity.**

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Marion Joncic
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* **Former group members**

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