Efficiency of STERRADs on Prions

In vitro & *In vivo* studies

Pascal CLAYETTE

SPI bio bertin

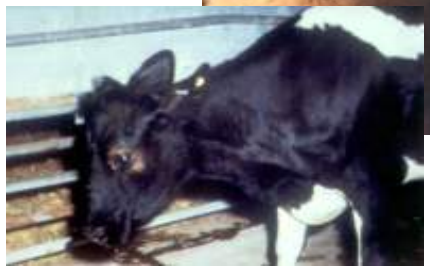
ASP ADVANCED STERILIZATION PRODUCTS® a Johnson & Johnson company

SMP GmbH Prüfen Validieren Forschen

Transmissible Subacute Spongiform Encephalopathies

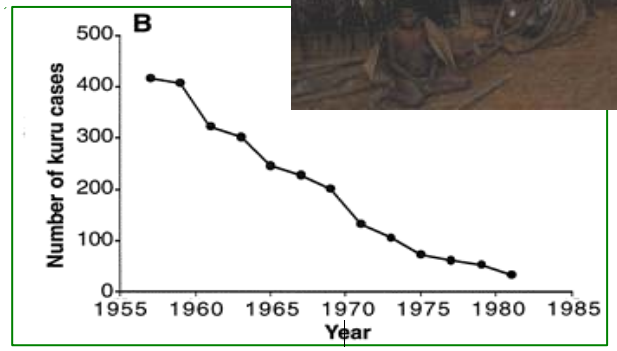
ANIMALS

- Natural Scrapie in Sheep and Goat – Europa – 1732
- Bovine Spongiform Encephalopathy (180 000 cases – more than 900 000 contaminated bovine in food) – UK – 1985
- Feline Spongiform Encephalopathy (FSE) – 1990
- Transmissible mink encephalopathy (TME)
- Chronic Wasting Disease (CWD) – classified in TSE since 1978



HUMANS

- Creutzfeld-Jakob Disease – Europa – 1920
- Kuru – New Guinea – 1951
- Gerstmann – Sträussler – Scheinker Syndrome
- Fatal Insomnia
- Variant (vCJD) – 1995



Characteristics of Transmissible Subacute Spongiform Encephalopathies (TSE)

- Transmissible but not contagious
- Long asymptomatic incubation period
  - Median age for clinical evolution : 62 *vs.* 29 years for vCJD
  - Duration of the disease also different, 14 months for vCJD
- Neurodegeneration of the central nervous system (CNS)
- Subacute course of the symptomatic phase
- Always fatal
- Lesions are identifiable only in the CNS
- No immune reaction, no inflammation, no demyelination of the CNS
- No virus, no microorganism can be evidenced in the CNS despite high infectivity titres in the brain

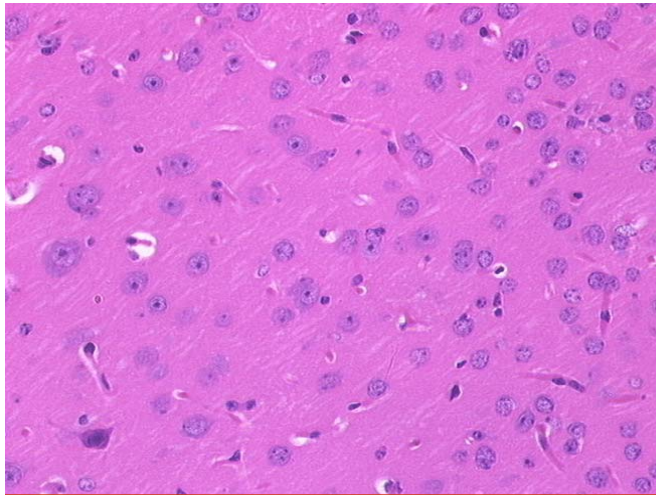
Clinical & biological Diagnosis

- Rapid dementia
- Ataxia and incoordination (no control of movements)
- Myoclonus (muscular spasms)
- Psychiatric symptoms (Depression) _vCJD specific
- No classical clinical manifestations associated with infectious diseases (neither fever nor flu-like state)
- No specific blood or cerebrospinal fluid disorder
- No detected response against infection or agent replication (Absence of non invasive diagnostic test for preclinical & symptomatic phases)

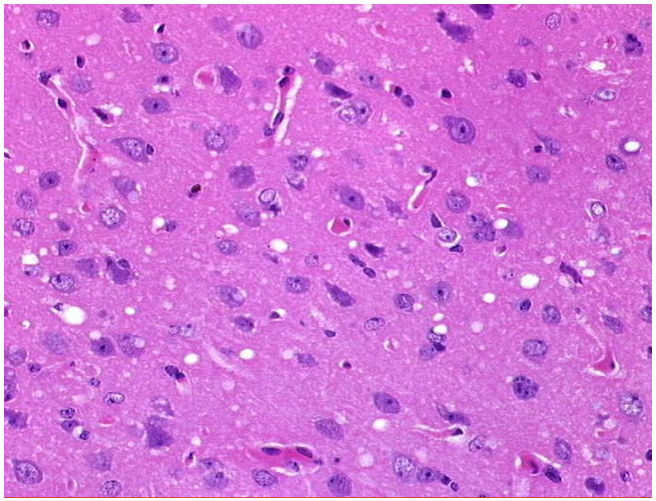
TSE Neuropathology: Similar for all infected species

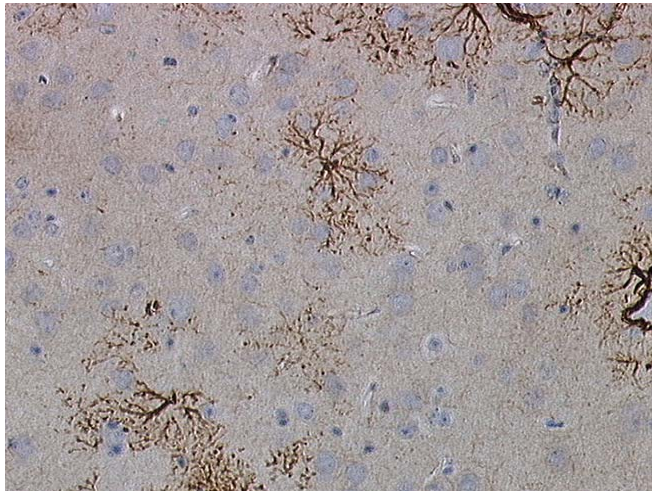
- **Neuronal death**
- **Spongiosis** (neuropile)
- **Astrogliosis**

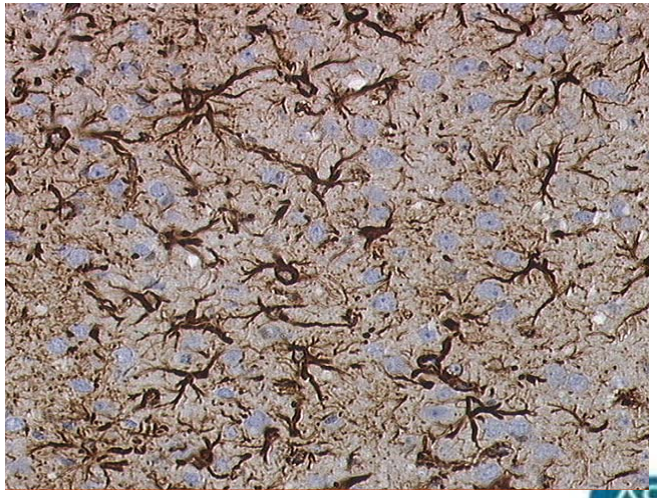
Uninfected Control



TSE-affected hamster

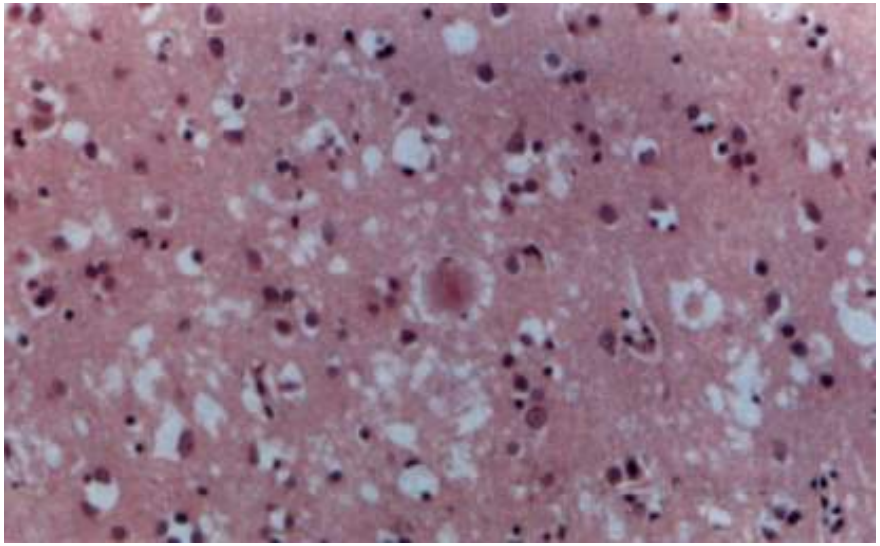






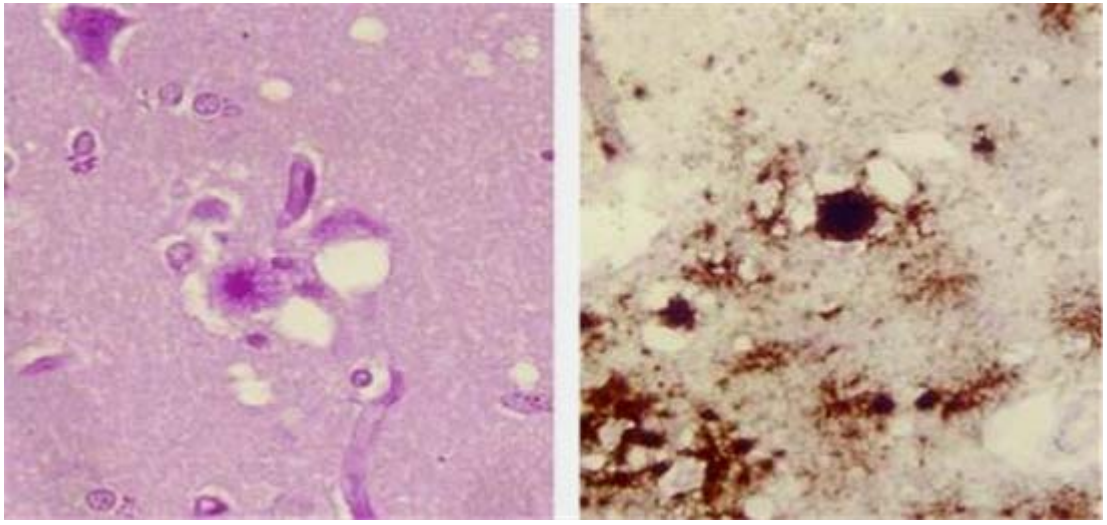
BSE & vCJD neuropathology

- **Spongiosis**



- **PrP amyloid deposit (Floride plaques)**

  Major and specific in vCJD



Brandel, 2001

The Prion hypothesis

- **Protein that accumulate specifically during TSEs proportionally to the infectious titre**

  Prion Protein

- **Prions are composed only of PrPsc molecules**

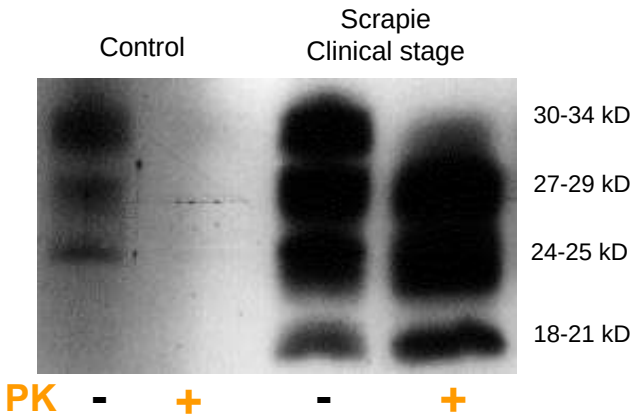
  (defined by PK resistance)

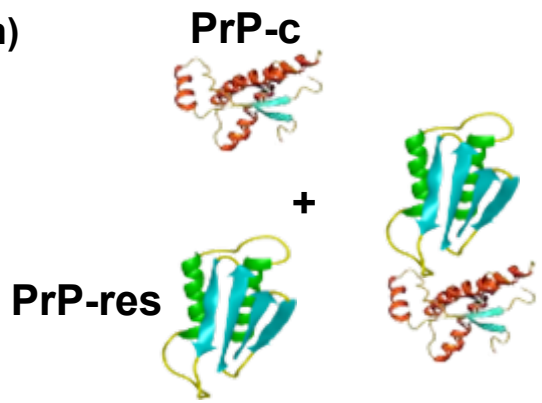
  PrPsc = PrPc derivative

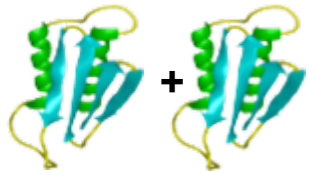
- **PrP-res which accumulates in infected individuals is derived from the PrP-c of the host**

  (not from the PrPres in the inoculum)

  Transconformation model





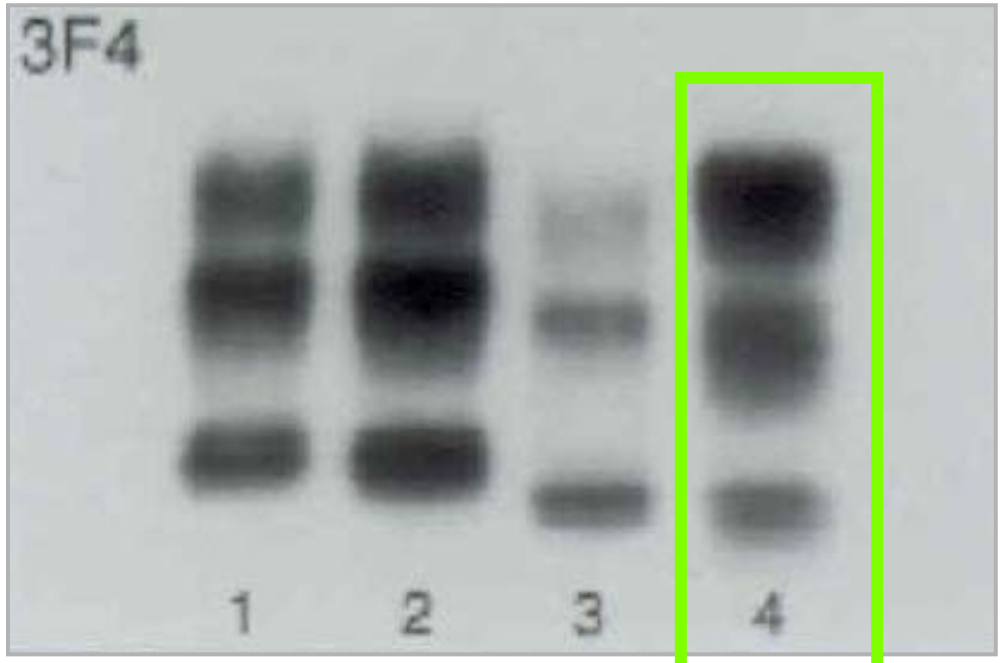


The Prion Hypothesis

- **The diversity of strains is « carried » by the tertiary structure of PrP^{sc}**

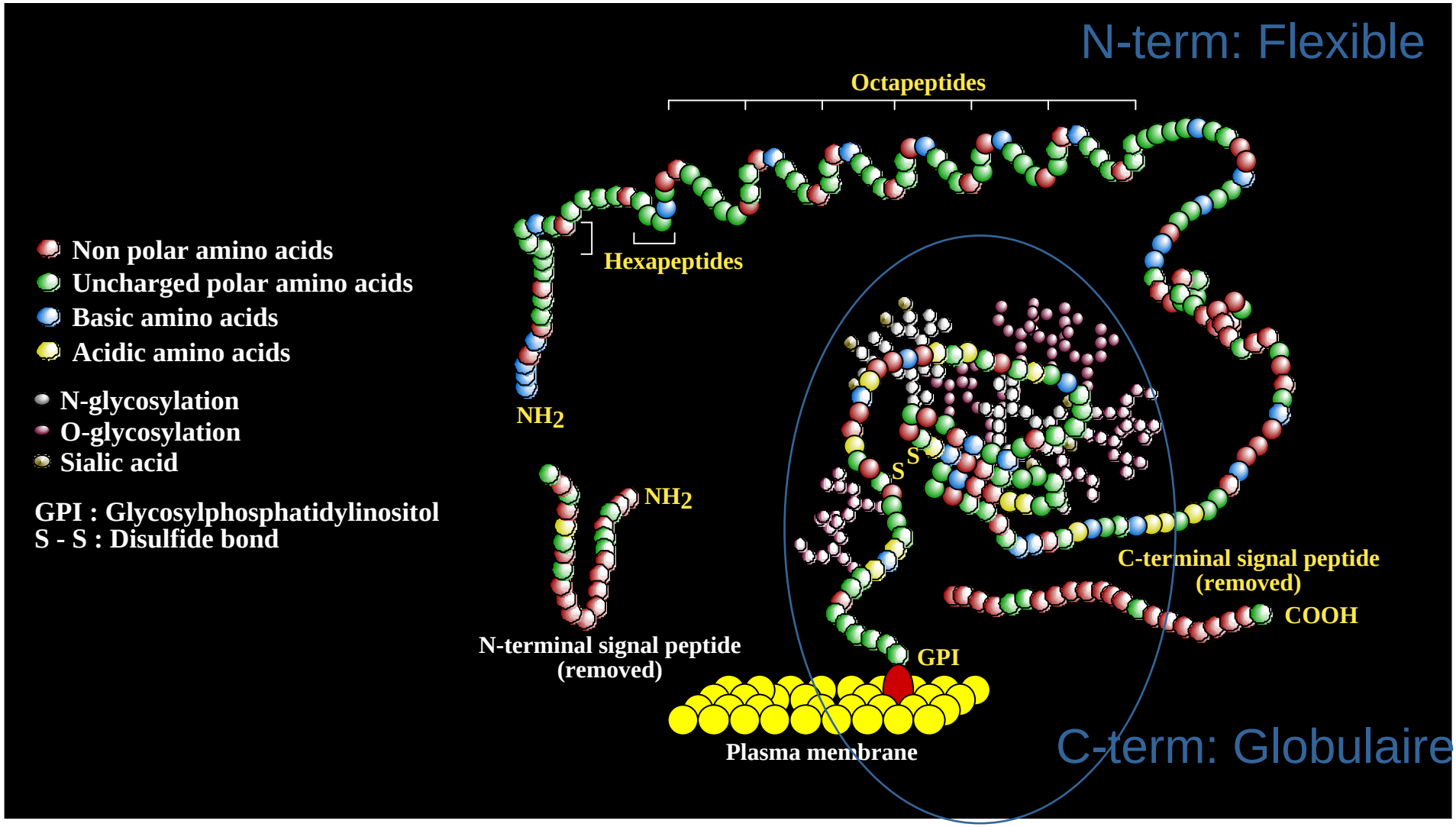
  Role of the « codon 129 »

Electrophoretic profil of PrP-res from different forms of CJD

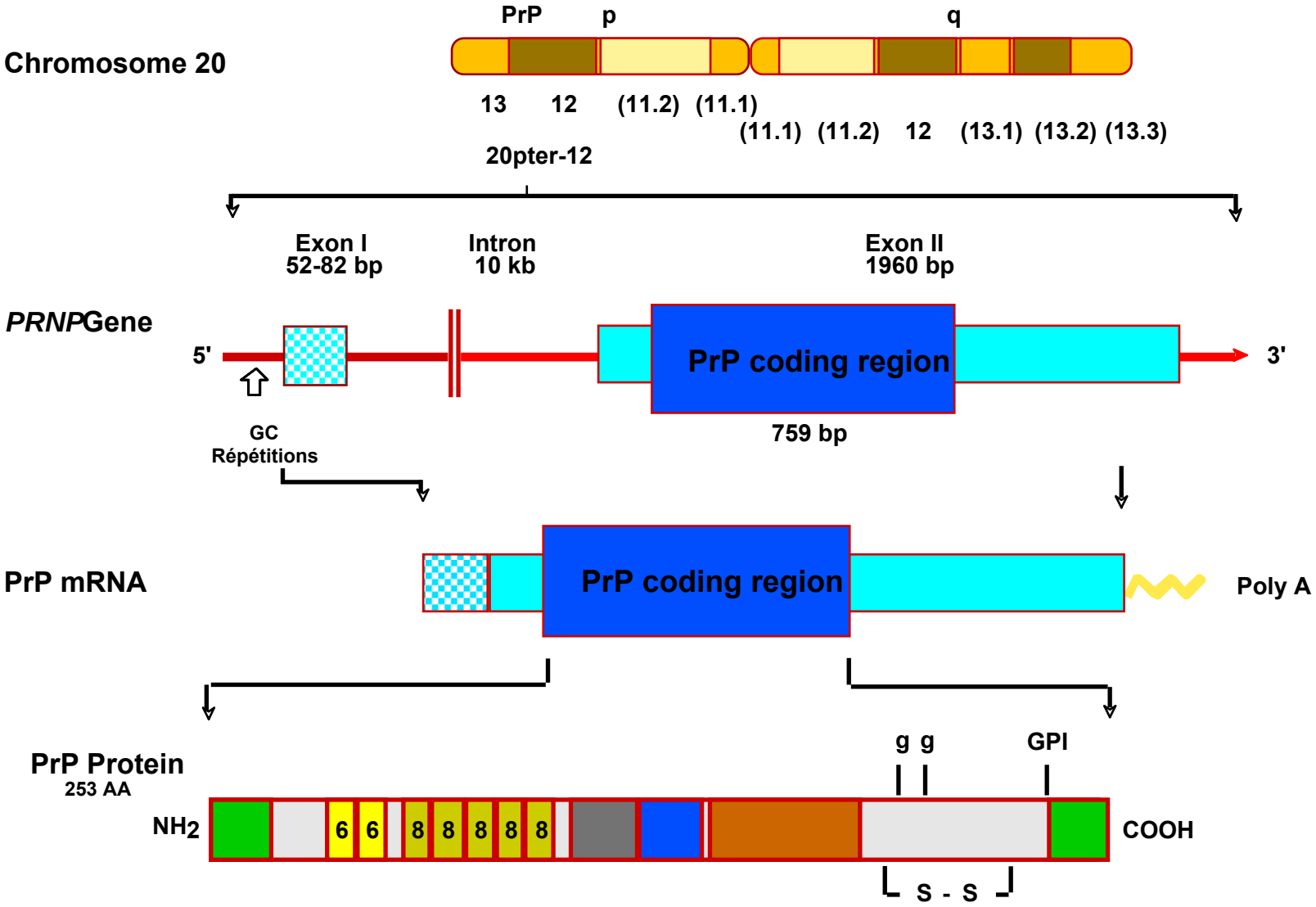


Hill et al, 1996

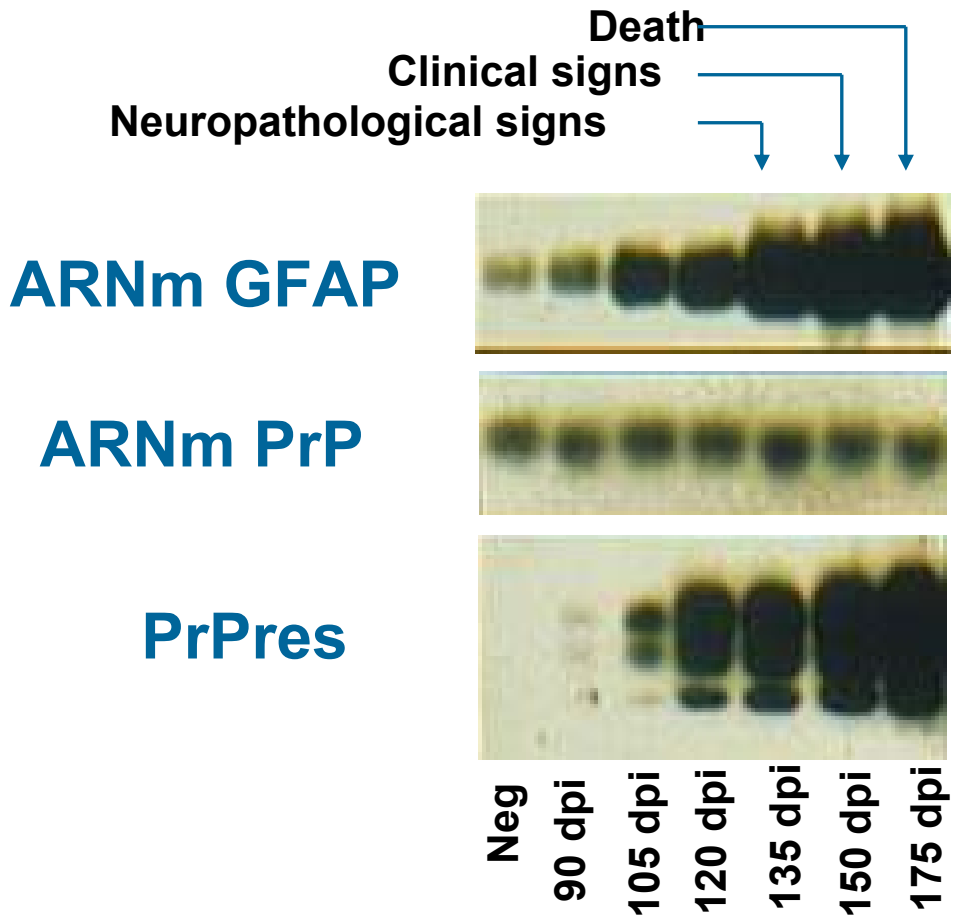
The Prion protein



The PrP gene (human)



Expression of the PrP gene



TSE: Main issues

- **Peripheral distribution of TSE agent**
- **Absence of validated non-invasive diagnostic method, particularly during the asymptomatic phase**
- **Extreme resistance to classical decontamination processes**

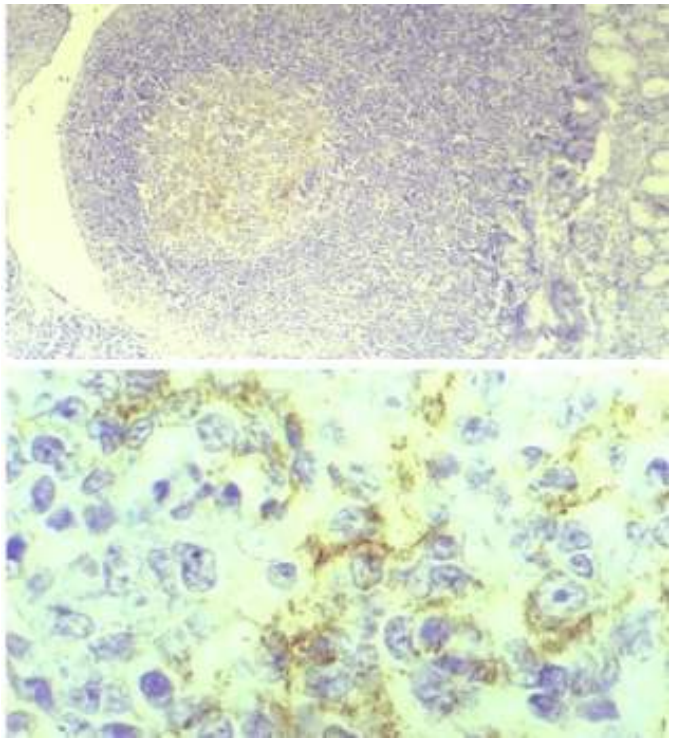
TSE agents: Biological risk

Distribution of Infectivity in Human TSE

	Spleen	Lymph Node	Tonsil
Healthy Controls	0/20	0/20	0/20
Neurodegenerative diseases except TSE	0/5	0/5	0/5
fCJD	0/7	0/7	0/7
sCJD	0/45	0/45	0/44
vCJD	15/15	15/15	15/15

Collinge et al, Will et al, Ironside et al, 1999

vCJD : Tonsil - biopsy



Hauw, 2001

vCJD: additional risk related to peripheral distribution of the vCJD agent _ lymphoreticular system / digestive tractus

Blood & Urine prions, Muscle prions

A Protease-resistant Prion Protein Isoform Is Present in Urine of Animals and Humans Affected with Prion Diseases*

Received for publication, May 28, 2001, and in revised form, June 14, 2001
Published, JBC Papers in Press, June 21, 2001, DOI 10.1074/jbc.C100278200

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Journal of Virological Methods xxx (2005) xxx–xxx

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Prion urine comprises a glycosaminoglycan-light chain IgG complex that can be stained by Congo red

Michele Halimi, Yael Dayan-Amouyal, Zehavit Kariv-Inbal, Yael Friedman-Levi, Tehila Mayer-Sonnenfeld, Ruth Gabizon*,[1]

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Received 2 September 2005; received in revised form 28 October 2005; accepted 8 November 2005

Preclinical deposition of pathological prion protein PrP^{Sc} in muscles of hamsters orally exposed to scrapie

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[1]Robert Koch-Institut, P26 – Pathogenese und Diagnostik Transmissibler Spongiformer Enzephalopathien, Berlin, Germany.
[2]Universitätsklinikum Göttingen, Institut für Neuropathologie, Göttingen, Germany.

The Journal of Clinical Investigation http://www.jci.org Volume 113 Number 10 May 2004

TSE agents: inactivation

Dry heat

- 180°C during 24 hours: residual infectivity is still detectable
- 320°C during 1 hour: residual infectivity is still detectable
- 600°C during 15 minutes: residual infectivity is still detectable

Probably related to the nature of the Prion agent i.e. Protein

TSE and healthy risk

Possible interventions

- **Sourcing:** Not easy
- **Screening:** Technical limit, today
- **Removal:** Variability and extrapolation not possible
- **Inactivation:** Often incompatible with materials

Material decontamination

New HICPAC Guidelines
« Special prion reprocessing »

- **NaOH and steam sterilisation** (e.g. 1 N NaOH, 121°C 30 min.)
- **134°C for 18 minutes** (prevacuum autoclaving)
- **132°C for 60 minutes** (gravity autoclaving)

Not compatible with heat-sensitive materials
Conditions of use are of importance

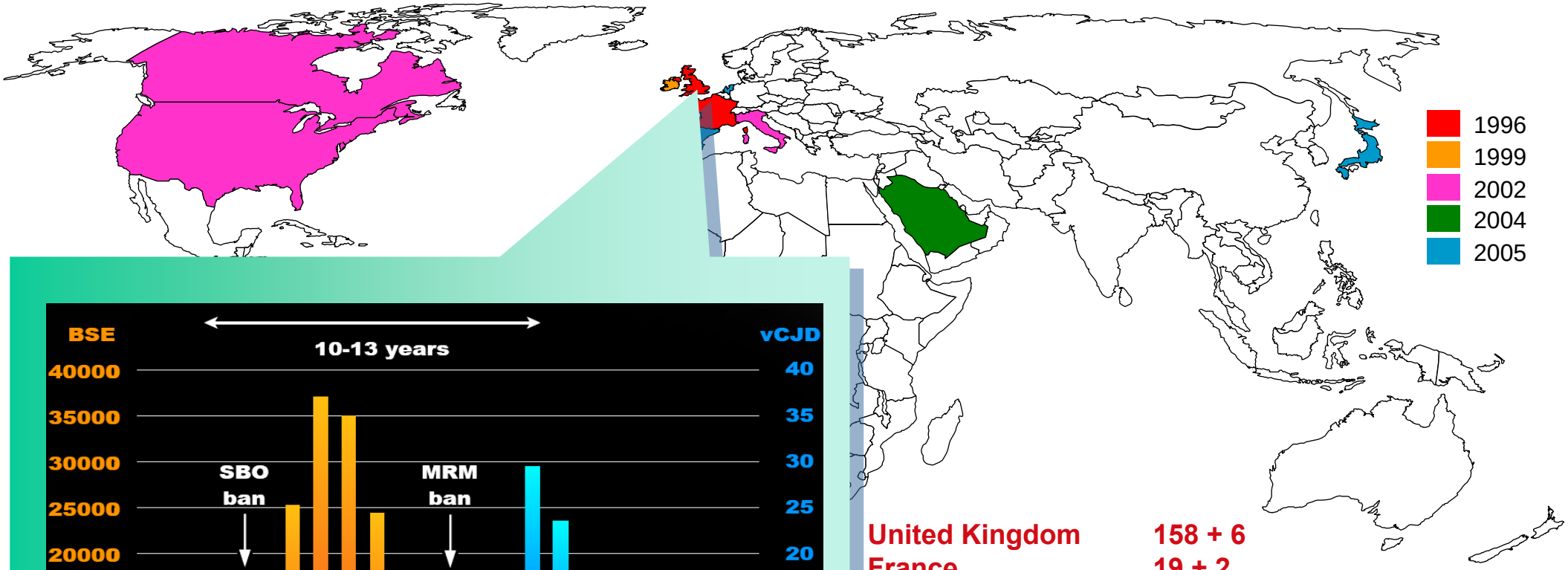
Material decontamination

Sodium hydroxide example

- **The 1 M NaOH treatment at room temperature totally decreases the 4 log10 PrPres titre of a mouse adapted BSE strain**
  RF > 3.5 log10
- **The 0.5 M NaOH treatment at +15°C decreases efficiently the PrPres titre but no complete effects was obtained**
  RF ~ 3 log10
- **The 0.5 M NaOH treatment at +4°C are only slightly efficient**
  RF ~ 1.5 log10

Treatment duration: 1 hour
Western blot

vCJD in the world



United Kingdom	158 + 6
France	19 + 2
Ireland	3 + 1
Italy	1
The Netherland	1 + 1
Portugal	1
Spain	1
USA	2
Canada	1
Japan	1

« Prions risk »

The incidence of vCJD in the UK is decreasing but there remain uncertainties for future numbers of cases.

While other countries have not been involved to the same degree, France continues to identify new cases and new countries have been affected.

With the control of BSE in cattle and the precautions taken to prevent BSE infected material to enter the human food chain, there remains the issue of controlling secondary human-to-human transmission.

Transmission via surgery remains a concern, but to date there is no evidence that it has actually occurred.

Need to identify new methodologies efficient against prions and compatible with surfaces of instruments

STERRAD Studies

- To compare efficacy of different generations of low-temperature sterilizer systems versus Steam on Prions: STERRAD® 100S, STERRAD® NX™ and STERRAD® 100NX™
- To measure possible interactions with alkaline or enzymatic detergents

Laboratories conducting the studies

In vivo study – Directed by Klaus Roth
SMP Gmbh (Tübingen, Germany) in collaboration with :

- University of Tübingen
- Federal Reference Center for Virus Diseases of Animals

In vitro study – Directed by Pascal Clayette, PhD
SPI-BIO, Neurovirology laboratory (Fontenay-aux-Roses, France)

- SPI BIO is a spin-off of CEA, one of the reference research center for prion diseases in France
- Pascal Clayette was a close collaborator of late Pr Dominique Dormont, scientist involved in Prions research and expert for Afssaps and EMEA

Methods and Method Combinations Tested

Reference Methods

- Steam (134°C, 18 minutes)
- Sodium hydroxide (1N, 1 h room temperature) followed by steam (134°C, 18 minutes)

STERRAD® Sterilization Systems

- Gas Hydrogen Peroxide: STERRAD®100S GMP, 100S, NX™, 100NX™
- Liquid Hydrogen Peroxide (59% at room temperature)

Detergents

- Enzymatic, at 37°C, alone or followed by:
  - Steam
  - STERRAD®
- Alkaline A and B, at 55°C and 70°C, alone or followed by:
  - Steam
  - STERRAD®

Phases of the Study

2002 - 2005 Bioassay

- **STERRAD® 100S / reference methods**
  - **Steam and Steam + NaOH**
  - **STERRAD® 100S long cycle (1 cycle and 2 consecutive cycles)**
  - **Comb. of Steam or STERRAD® 100S + alkaline or enzymatic detergents**

2005 - 2007 Bioassay

- **STERRAD® NX™ vs. STERRAD® 100S**
  - **STERRAD® NX™ Advanced cycle (1 cycle and 2 consecutive cycles)**
  - **Combination of STERRAD® 100S or NX™ + alkaline detergents**

2007 *In vitro* tests on various strains + support material

- **STERRAD® 100S, STERRAD® NX™ and STERRAD® 100NX™ vs. steam**
  - **STERRAD® 100NX™ Standard and Flex cycles**
  - **STERRAD® NX™ Advanced cycle**

Prion strains used

263K Scrapie strain (*in vivo* and *in vitro*)

- **Hamster-adapted 263K Scrapie strain**
- **A reference strain for the evaluation of new processes able to eliminate/inactivate prions**

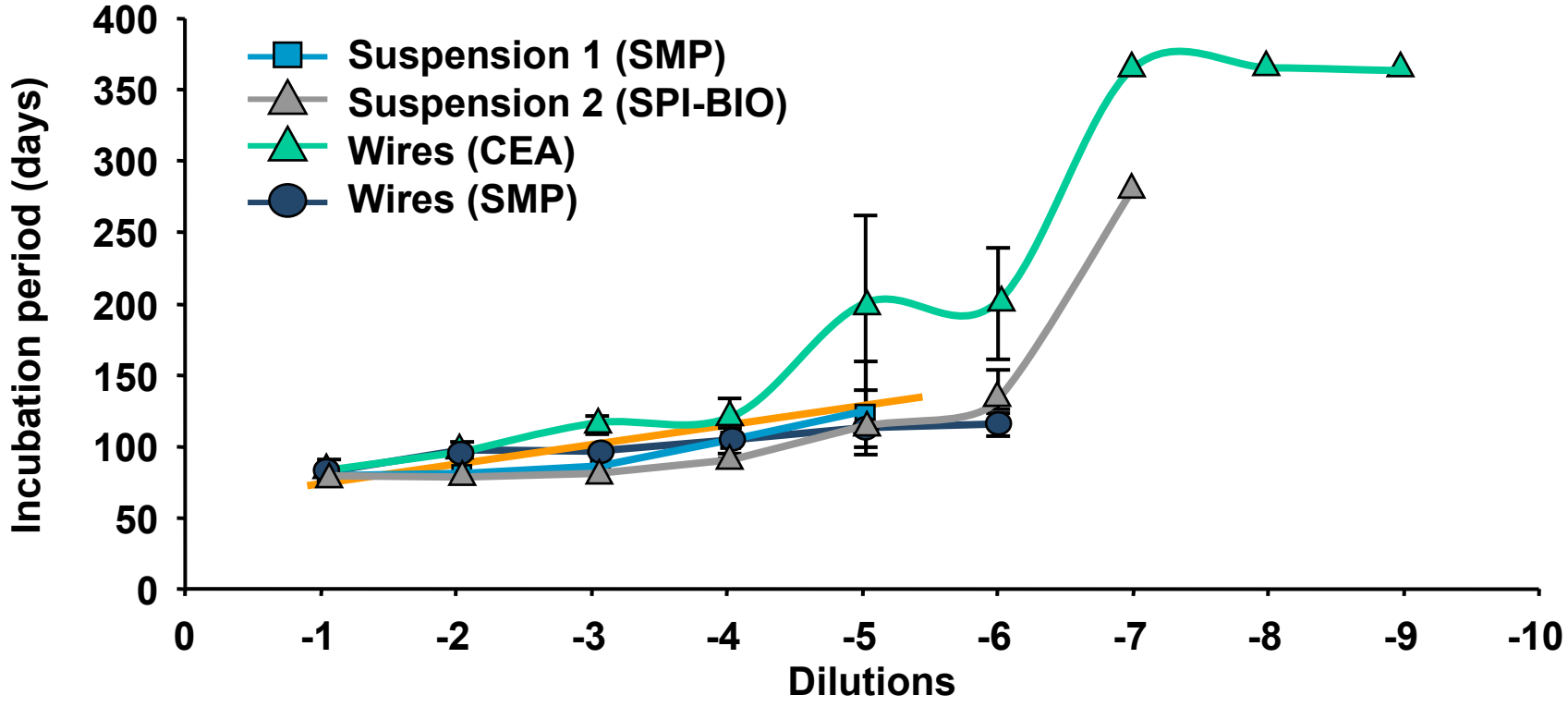
“Human” strains: BSE and vCJD (*in vitro*)

- **Mouse-adapted 6PB1 BSE strain**
- **vCJD strain**

Bio-assay: Methodology

- **Stainless steel wires (1.4301; Flechsig et al., 2001)**
  Ø : 0,3 mm / L : 5 mm
  - **Incubated in 10% brain homogenate in phosphate tampon for 16 h**
  - **Dried at room temperature**
  - **Treated with the different processes (except for positive control)**
  - **Dried at room temperature before implantation**
- **Implantation**
  - **Using an injection needle**
  - **Use of a stereotaxic instrument**
  - **Insertion in the brain of the anesthetized hamster**
- **Identical position of all wires**
- **Clinical and biochemical (Western Blot + PET Blot) monitoring**

Bio-assay: Correlation Between Infectious Dose and Incubation Period



An incubation delay of approximately 12 days corresponds on average to a reduction of 1 log of the infection level.

After 200 days, only residual infectivity detected.

Results 1: Control Groups

	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
Negative control (wires exposed to 10% normal brain homogenate)	0%	606 ± 118	-	-
Positive control (wires exposed to 10% 263K-infected brain homogenate)	100%	83 ± 3	-	-
10% 263K-infected brain homogenate	100%	78 ± 2	-	-
Wires implanted for only 5 minutes	100%	101 ± 5	18	1.5
Steam 134°C 18 min	50%	428 ± 103	345	≥ 5-6
NaOH 1N 1h RT + Steam 134°C 18min	28%	554 ± 197	474	≥ 5-6

- **Implantation: no effect on animals life expectancy**
- **5 minutes insertion: sufficient to infect the animals**
- **Reference method: results in accordance with data previously published** (Vadrot et Barbor, 2006)

Results 2: Hydrogen Peroxide (STERRAD® 100S vs. Liquid H_2O_2)

	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
59% H_2O_2 for 10 min at room temperature	50%	443 ± 140	360	≥ 5-6
STERRAD® 100S GMP 1 long cycle	100%	96 ± 4	14	1.1
STERRAD® 100S 1 long cycle	100%	99 ± 6	16	1.3
STERRAD® 100S 2 cons. long cycles	100%	104 ± 8	22	1.8
Steam 134°C 18 min	50%	428 ± 103	345	≥ 5-6
NaOH 1N 1h RT + Steam 134°C 18min	28%	554 ± 197	474	≥ 5-6

- Hydrogen peroxide solution: significant reduction of infectivity
- Comparable effect (moderate) of STERRAD® 100S GMP and 100S
- STERRAD® 100S: 2 cons. cycles > 1 cycle
- Low efficacy of STERRAD® compared to reference method

Results 3: Enzymatic and Alkaline Detergents

	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
2% enzymatic detergent (37°C, 10 min)	100%	95 ± 0	13	1.1
100% enzymatic detergent (37°C, 30 min)	100%	94 ± 2	12	1.0
100 % enzymatic detergent (37°C, 24 h)	100%	93 ± 1	11	0.9
1% alkaline detergent A (55°C, 10 min)	11%	446 ± 153	363	≥ 5-6
1% alkaline detergent B (55°C, 10 min)	0%	524 ± 42	441	≥ 5-6
Steam 134°C 18 min	50%	428 ± 103	345	≥ 5-6
NaOH 1N 1h RT + Steam 134°C 18min	28%	554 ± 197	474	≥ 5-6

- Enzymatic detergent: moderate efficacy
- Alkaline detergents: significant reduction of infectivity

Results 4: Enzymatic Detergent Combined With Steam or STERRAD® 100S

	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
2% enzymatic detergent (37°C, 10 min)	100%	95 ± 0	13	1.1
Steam 134°C 18 min	50%	428 ± 103	345	≥ 5-6
2% enzymatic detergent (37°C, 10 min) + steam (134°C 18 min)	100%	131 ± 17	48	4.0
STERRAD® 100S GMP 1 long cycle	100%	96 ± 4	14	1.1
2% enzymatic detergent (37°C, 10 min) + STERRAD® 100S GMP 1 long cycle	100%	111 ± 12	29	2.4
100% enzymatic detergent (37°C, 30 min)	100%	94 ± 2	12	1.0
STERRAD® 100S 2 cons. long cycles	100%	104 ± 8	22	1.8
100 % enzymatic detergent (37°C, 30 min) + STERRAD® 100S GMP 2 cons. long cycles	67%	211 ± 125	128	≥ 5-6

- Enzymatic detergent + steam > enzymatic detergent alone
- But steam alone > steam + enzymatic detergent
- Enzymatic detergent + STERRAD® 100S 1 or 2 long cycles: Additive effects but infectivity still detected

Results 5: Alkaline Detergent Combined With STERRAD® 100S

	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
1% alkaline detergent A (55°C, 10 min)	11%	446 ± 153	363	≥ 5-6
1% alkaline detergent A (55°C, 10 min) + STERRAD® 100S GMP 1 long cycle	0%	496 ± 64	413	≥ 5-6
1% alkaline detergent A (55°C, 10 min) + STERRAD® 100S GMP 2 cons. long cycles	0%	540 ± 30	457	≥ 5-6
1% alkaline detergent B (55°C, 10 min)	0%	524 ± 42	441	≥ 5-6
1% alkaline detergent B (55°C, 10 min) + STERRAD® 100S 1 long cycle	0%	540 ± 13	457	≥ 5-6
1% alkaline detergent B (55°C, 10 min) + STERRAD® 100S GMP 2 cons. long cycles	0%	552 ± 0	476	≥ 5-6

Alkaline detergents + STERRAD® 100S, 1 or 2 long cycles: significant effects with not detected infectivity

Results 6: STERRAD® NX™

	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
STERRAD® NX™ 1 Advanced cycle	0%	570 ± 18	487	≥ 5-6
STERRAD® NX™ 2 cons. Advanced cycle	0%	574 ± 0	491	≥ 5-6
Steam 134°C 18 min	50%	428 ± 103	345	≥ 5-6
NaOH 1N 1h RT + Steam 134°C 18min	28%	554 ± 197	474	≥ 5-6

- STERRAD® NX™ : Infectivity not detected
- STERRAD® NX™ : 1 Adv. cycle = 2 consecutive Adv. cycles

Results 7: STERRAD® NX™ With Pre-treatment

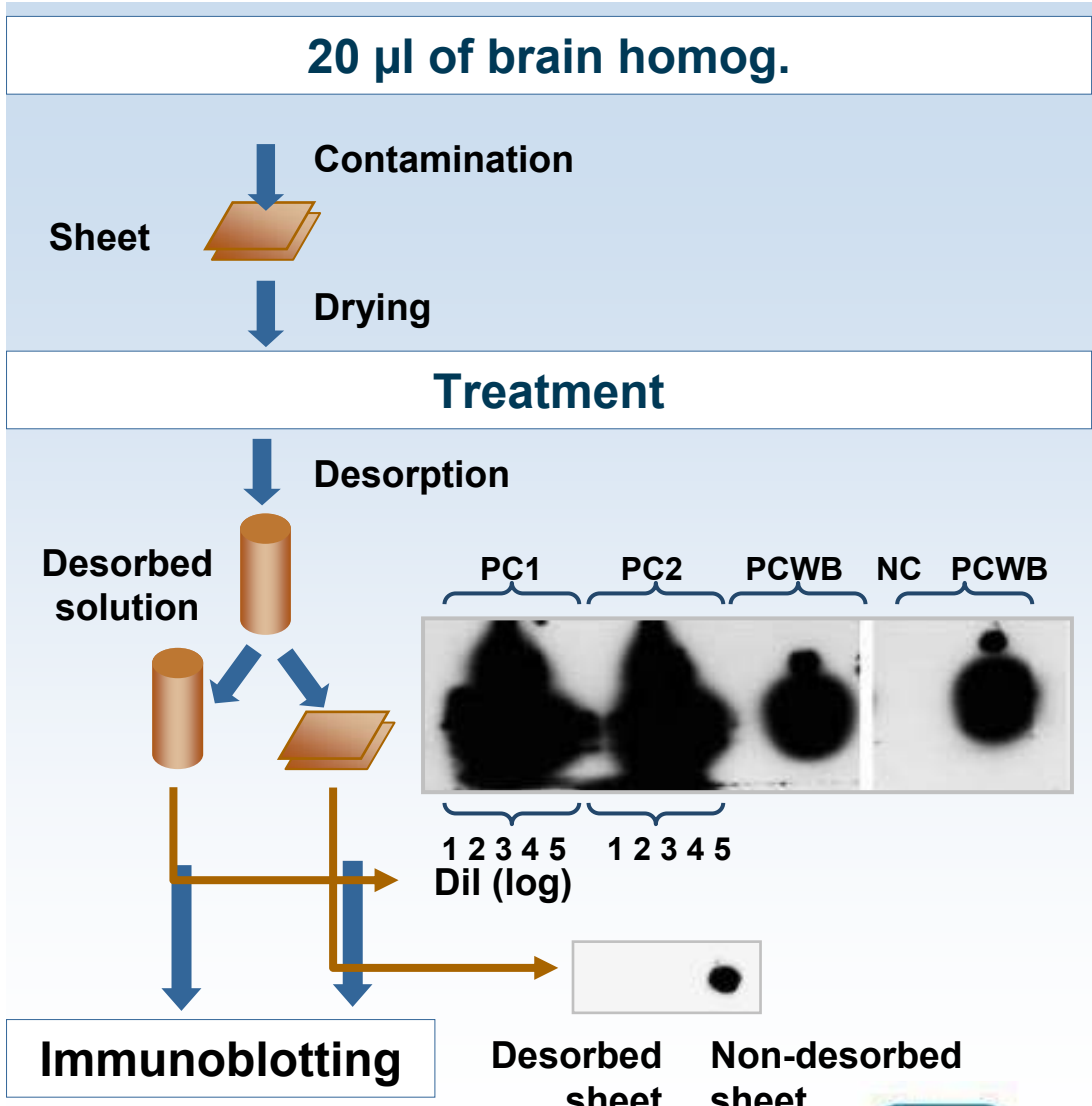
	Transmission Rate (%)	Incubation Period (days)	Incub. Delay (days)	RF
STERRAD® NX™ 1 Advanced cycle	0%	570 ± 18	487	≥ 5-6
1% alkaline detergent A (55°C, 10 min) + STERRAD® NX™ 1 Advanced cycle	0%	559 ± 22	476	≥ 5-6
1% alkaline detergent B (55°C, 10 min) + STERRAD® NX™ 1 Advanced cycle	0%	562 ± 16	479	≥ 5-6
Steam 134°C 18 min	50%	428 ± 103	345	≥ 5-6
NaOH 1N 1h RT + Steam 134°C 18min	28%	554 ± 197	474	≥ 5-6

- Alkaline detergents + STERRAD® NX™ : no antagonism
- Enzymatic detergent + STERRAD® NX™ : not tested

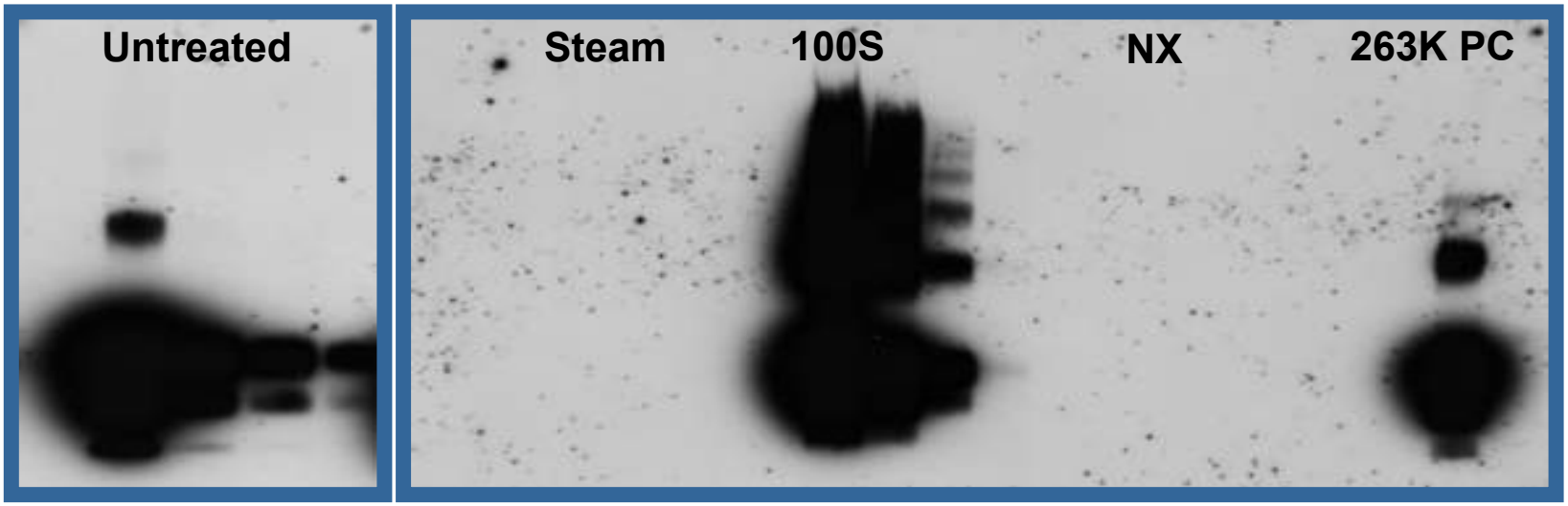
In vitro: Methodology

Sheets – 9 X 9 mm (Stainless Steel, Polypropylene, Polyethylene)

- 20 µL of inoculum
- Drying at room temperature for 16 hours
- Treatment with one of the tested processes (except positive controls)
- Desorption (Lemmer et al., 2004)
- Detection of residual PrPres on sheets
- Determination of PrPres titres by limit-dilution in desorption solutions



Results 8: 263K Strain With STERRAD ® 100S & STERRAD ® NX™



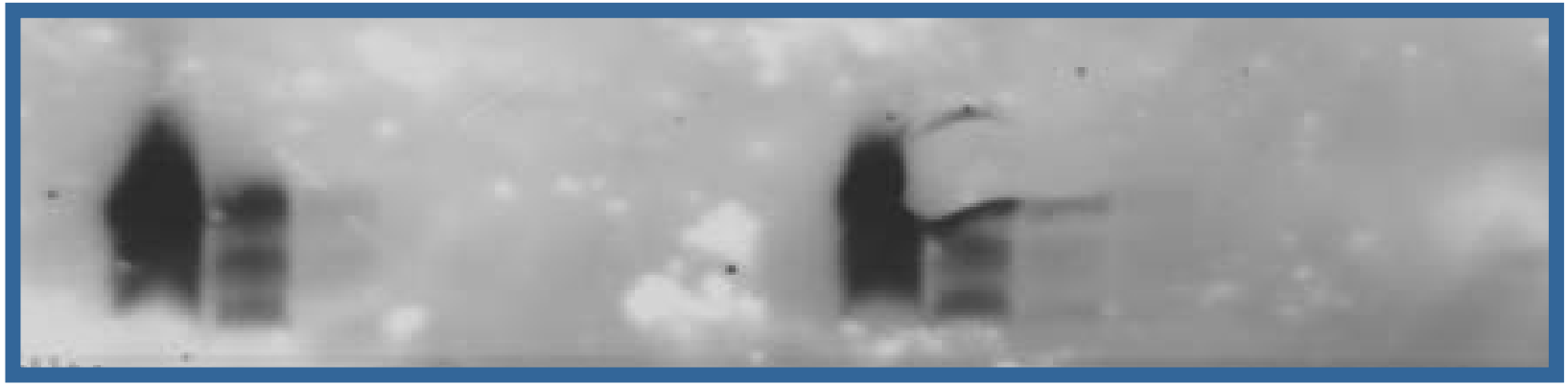
PC: Positive Control

Treatment	RF
Steam	≥ 5 log
STERRAD® 100S	2.5 log
STERRAD® NX™	≥ 5 log

- Coherent with *in vivo* results
- STERRAD® NX™ > STERRAD® 100S

Results 9: mBSE and vCJD Strains With STERRAD ® 100S & STERRAD ® NX™

vCJD



Contamination	RF	Steam	100S	NX
50X „overloaded“ 6PB1 BH	≥ 4.5	ND	ND	ND
vCJD BH	≥ 2.5	ND	ND	ND

ND: Not detected

- Steam: no PrPres signal (≥ 4.5 log)
- STERRAD® 100S and NX™: no PrPres signal

Results 10: Tests on heat-sensitive materials With STERRAD® NX™

263K strain

Dil. (log) 2 3 4 5 6 — Untreated; 0 1 2 3 4 — NX; WB PC

Dil. (log) 2 3 4 5 6 — Untreated; 0 1 2 3 4 — NX; WB PC

Contamination	Untreated	NX
Polypropylene (PP)	≥ 6.5	ND
Polyethylene (PE)	≥ 6.5	ND

0 = Neat

ND: Not detected

- **Identical efficiency of STERRAD® NX™ on the different surfaces tested**
  (stainless steel, polypropylene & polyethylene)

Results 11: 263K Strain With STERRAD® 100NX™

Dil (log)	2 3 4 5	2 3 4 5 6	0 1 2 3 4	WB PC
	Untreated	Standard	Flex	

Cycle	Untreated
“Standard”	≥ 5.5
“Flex”	≥ 5.5

- STERRAD® 100NX™ = STERRAD® NX™

Conclusions

STERRAD® 100S

- **STERRAD® 100S without cleaning: 1.3 log (1.8 log after 2 consecutive cycles)**
- **Alkaline detergent + STERRAD® 100S: ≥ 5-6 log and a transmission rate of 87.5% after 1 long cycle and 0% after 2 consecutive long cycles**

Conclusions (Continued)

STERRAD® NX™ (alone or combined with alkaline detergent)

- **No infectivity detected (bio-assay): ≥ 5-6 log**
- **Efficiency (*in vitro*) against 263K strain & "human" strains**

STERRAD® 100NX™

- **Identical efficiency (*in vitro*) against the 263K strain as compared to STERRAD® NX™**

Conclusions (Continued)

STERRAD® NX™ (*In vitro & in vivo*)

- **Effective in inactivating prions**
- **Just as effective as high temperature steam sterilization**

STERRAD® 100NX™ (*In vitro*)

- **Effective against prions** (not inactivation because only *in vitro*)
- **Just as effective as high temperature steam sterilization**

Prions and STERRAD®

Give in marriage and have several children…

C. Rogez-Kreuz
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V. Huyot
C. Aubenque
P. Clayette

Z-X. Yan
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