FDA Science Board

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Clearance of Transmissible Spongiform Encephalopathy Agent by Bovine Heparin Production

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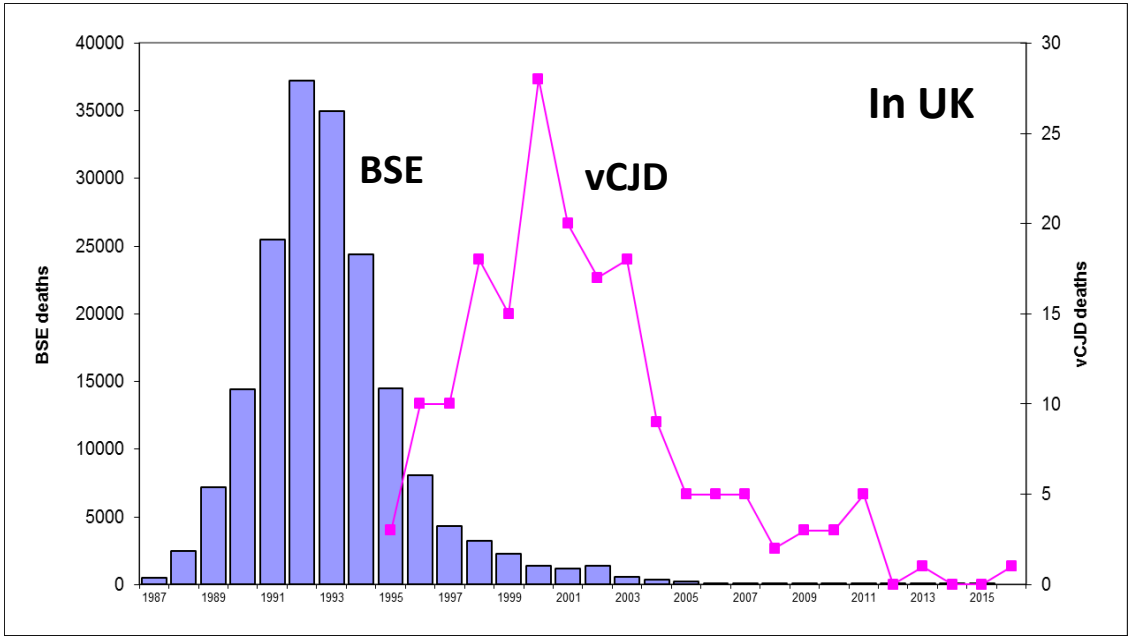
This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

BSE and vCJD

- Bovine heparin manufacturers discontinued product due to potential risk of Bovine Spongiform Encephalopathy (BSE) agent contamination
- BSE is a fatal neurological disease of cattle transmissible to humans (variant Creutzfeldt-Jakob disease)



228 cases of vCJD worldwide

Approaches to reduce risk of BSE agent contaminating biological products

- No accessible test for live animals
- To reduce BSE risk
  - Limit sources of bovine raw materials to safest possible
    - Low-risk countries (OIE/USDA)
    - BSE Surveillance Program with targeted testing at slaughterhouses
    - Low-risk cattle (traceable, never fed prohibited proteins, controlled herd with active BSE Surveillance Program, age <30 months at slaughter)
    - Low-risk tissues (intestines contain only small amounts of infectivity) excluding distal ileum
    - Removal of Specified Risk Materials (SRM: highest risk = CNS)
    - Prevent cross contamination of lower-risk tissues with SRM
  - Use manufacturing processes that reduce—physically remove or inactivate—infectivity in the raw materials

Goal of the project

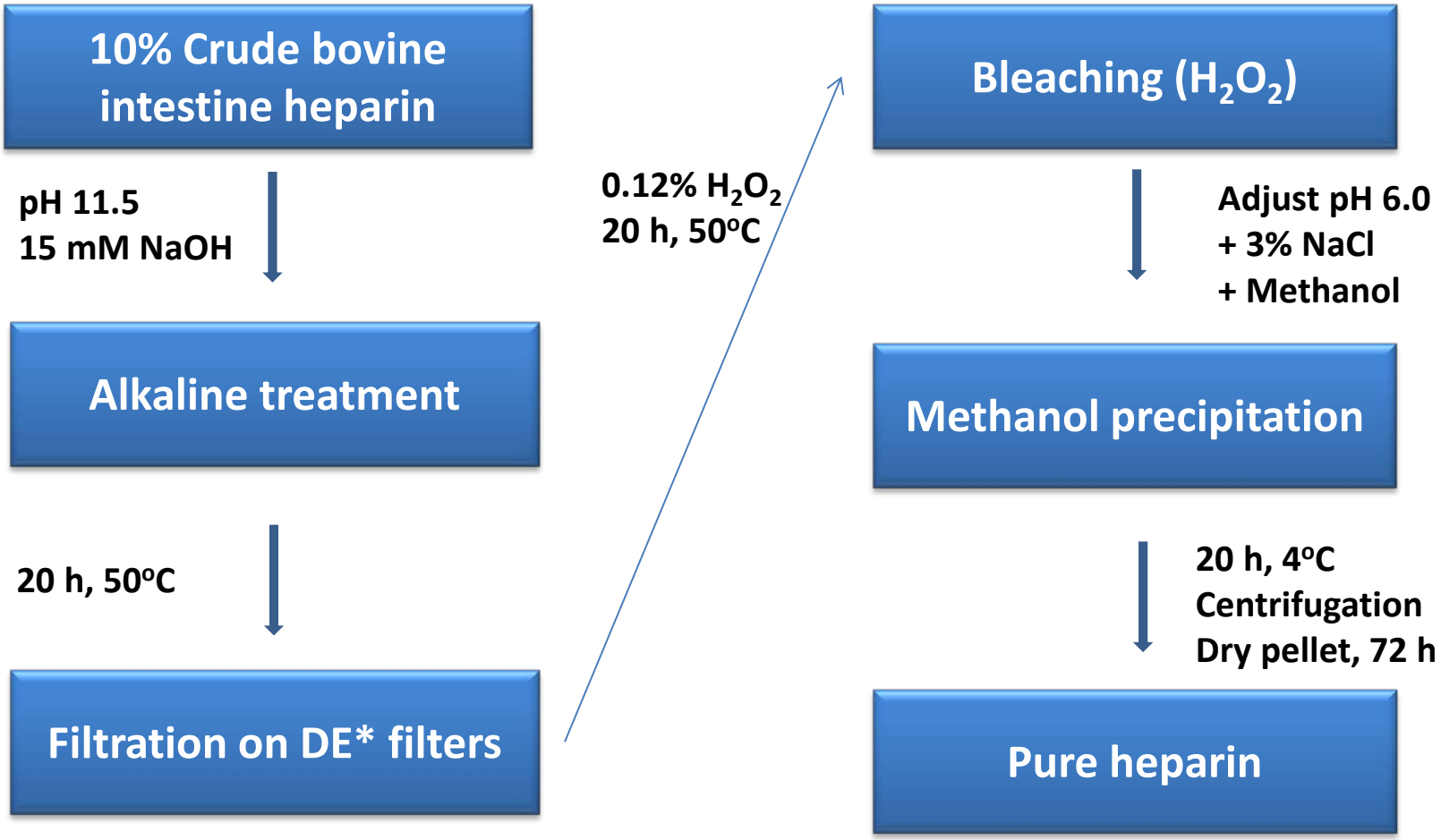
To assess whether the process for manufacturing heparin from crude heparin has an intrinsic capacity to reduce the risk of BSE contamination of the final product.

BSE clearance validation study for heparin manufacturing process

Study design

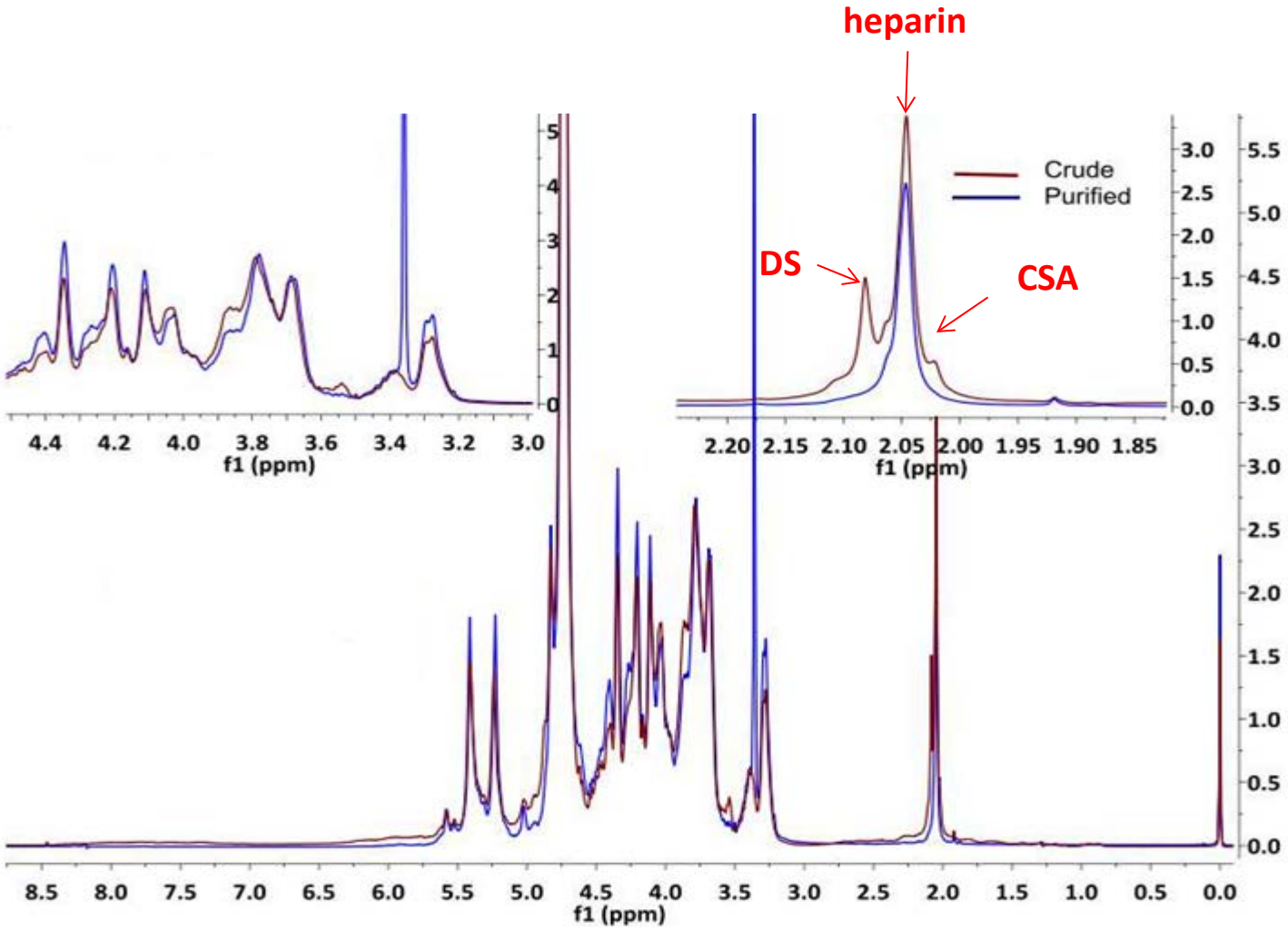
- Develop a model purification scheme for heparin using:
  - Published data
  - Generic process incorporating basic heparin purification steps
  - Not linked to any particular heparin manufacturer
- Test crude heparin spiked with scrapie-infected brain homogenate (scrapie agent: common surrogate for BSE agent)
  - Assay infectivity by animal bioassay
  - RT-QuIC in vitro assay to detect PrP^{TSE} (potential surrogate for infectivity bioassay)
- Repeat study with BSE-infected brain homogenate as more relevant agent spike

Heparin purification scheme



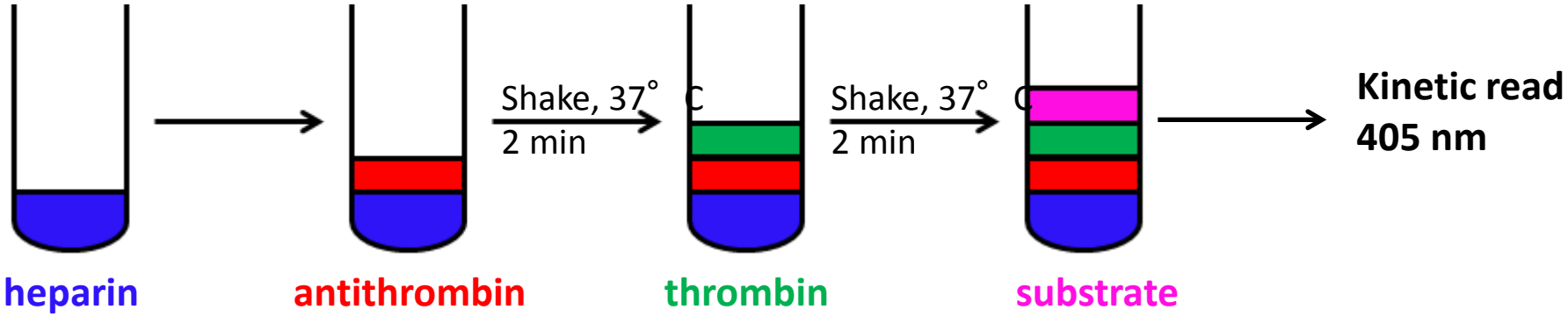
*Diatomaceous earth

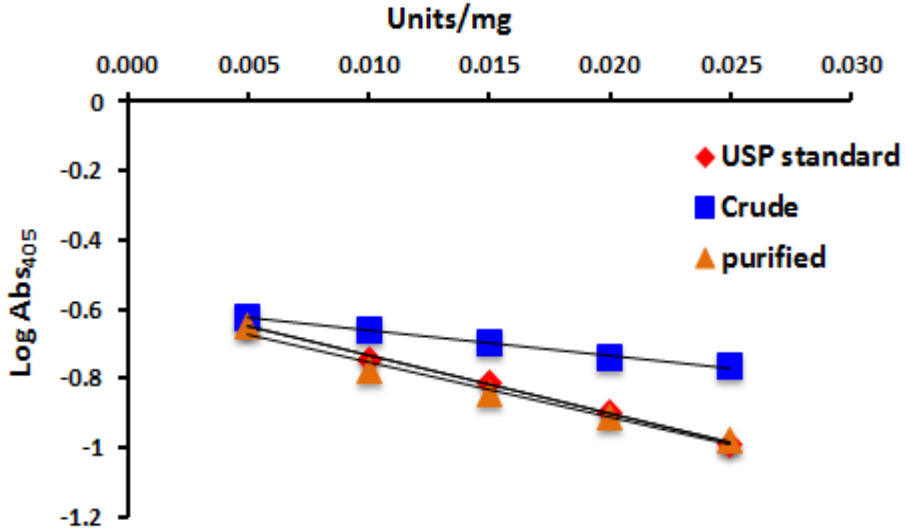
Proton NMR spectra of crude and processed heparin



^{1}HNMR – Confirms removal of heparin contaminants: Dermatan Sulfate (DS) and Chondroitin sulfate A (CSA)

Heparin anti-factor IIa potency assay





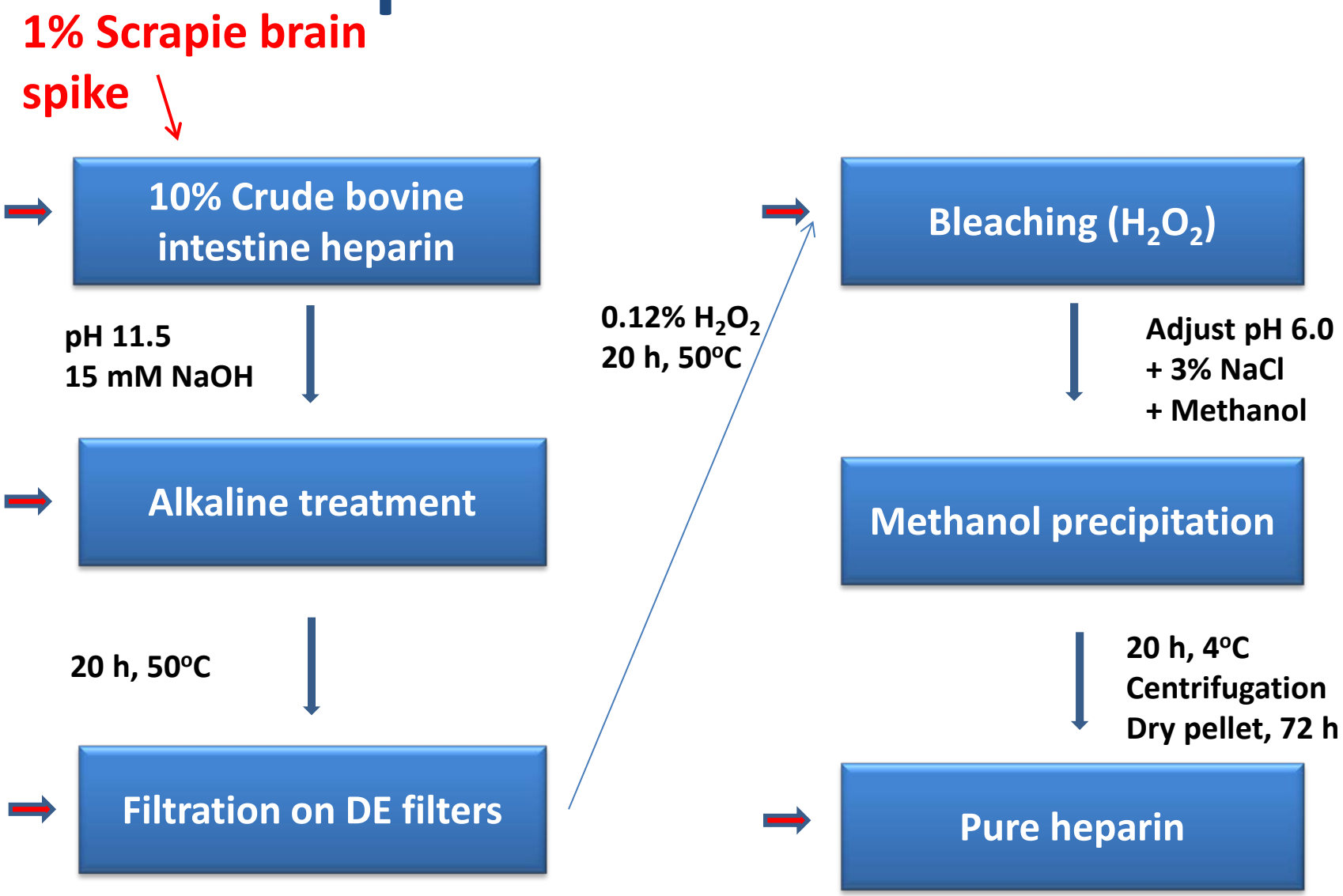
Potency = A x (S_T/S_S)

Heparin potency assay confirmed the quality of our purified heparin

Crude heparin spiked with scrapie-infected brain homogenate

- Scrapie agent (BSL-2) is a surrogate for BSE agent (BSL-3) and generally predicts BSE agent behavior
- Hamster infected with 263K strain of scrapie agent is a well-characterized animal model for TSE clearance validation studies
  - Highest infectivity titers of any animal model
  - Relatively short incubation periods
  - High levels of abnormal prion protein (PrP^{TSE})

Scrapie validation scheme



Hamster bioassay results

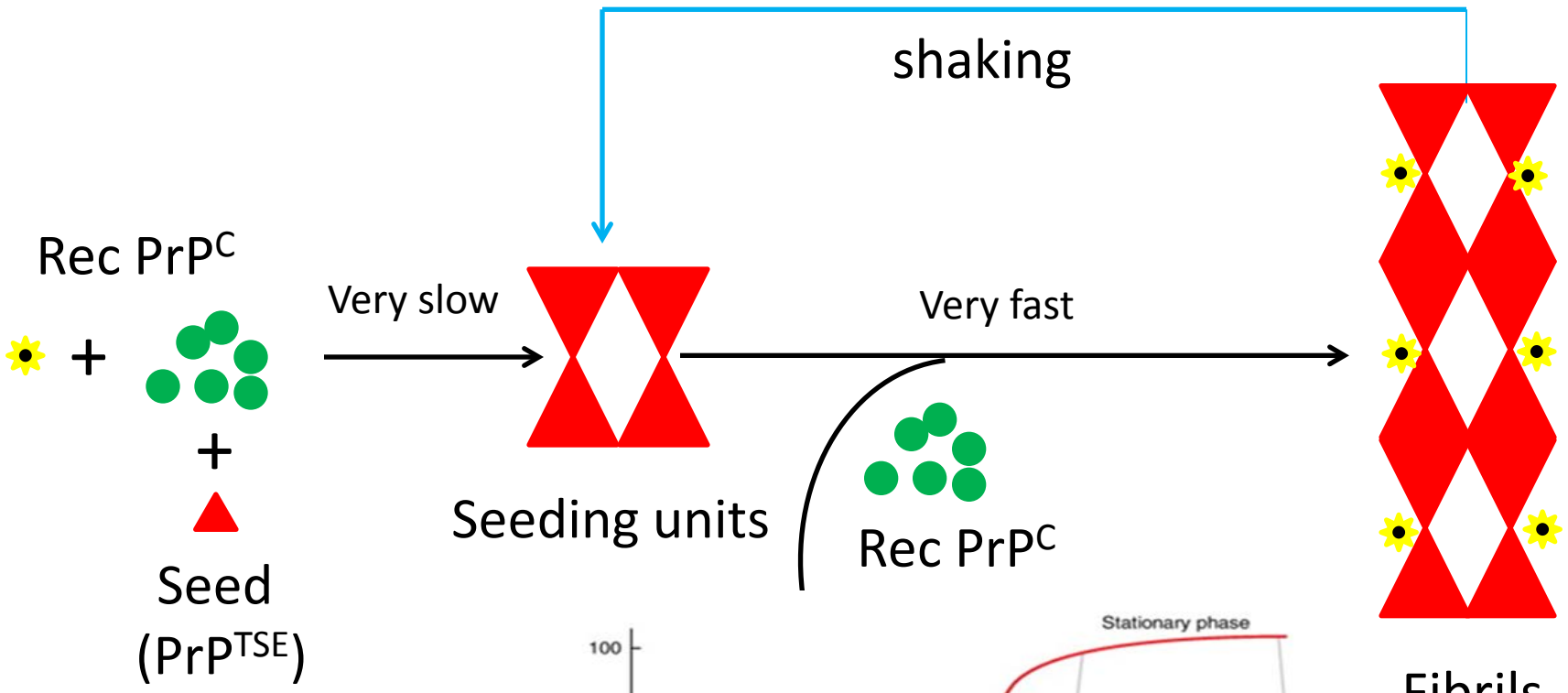
- Bioassays with aliquots of each step of the heparin purification process

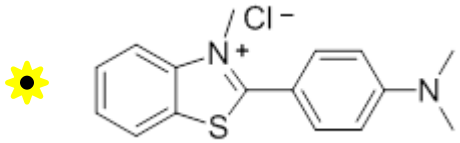
Dilutions	Heparin scrapie-spiked	NaOH treatment	DE filtration	H_2O_2 bleaching	Final product
10^{-3}	4/4 (91±11)*	6/6 (115±6)	8/8 (147±39)	8/8 (212±74)	11/11 (170±42)
10^{-4}	4/4 (97±2)	-	-	-	-
10^{-5}	4/4 (104±9)	-	-	-	-
10^{-6}	4/4 (119±6)	-	-	-	-
10^{-7}	4/4 (188±110)	-	-	-	-
10^{-8}	0/4 (>365)	-	-	-	-
10^{-9}	0/4 (>365)	-	-	-	-
$\log_{10}$ ID_{50}/g brain	**9.3**	**6.5±0.3**	**5.7±0.3**	**5.4±0.2**	**5.2±0.2**

*** Infected animals/total injected (average incubation period)**

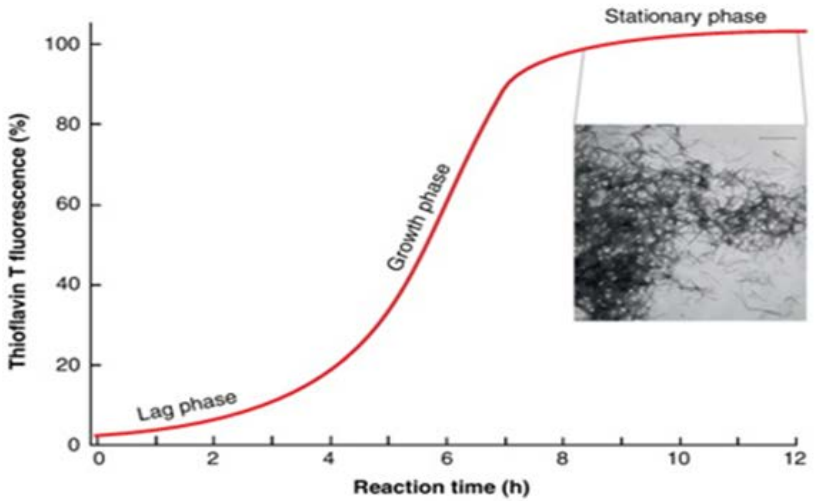
NaOH and filtration steps reduced scrapie infectivity

In vitro seeding assay for detection of PrP^{TSE}





Thioflavin T



Combined results

	RT-QuIC		
	log_{10} SD_{50}/g brain	Log10 removed	
Sample		Step	Total
Scrapie spike	12±0.4	-	-
NaOH treatment	9.6±0.4	2.4	2.4
DE filtration	8.6±0.2	1.0	3.4
H_2O_2 bleaching	8.1±0.2	0.5	3.9
Final product	8.1±0.2	0.0	3.9

Conclusions (scrapie study)

- Scrapie infectivity was reduced ~ 3.6 $\log_{10}$ by the first two purification steps
- RT-QuIC showed ~ 3.4-$\log_{10}$ reduction by the same steps
- RT-QuiC and bioassay demonstrated equivalent results
- RT-QuIC might replace animal bioassay when using scrapie agent

BSE spike study (FDA BSL3-ABSL3 labs)

- Same heparin purification scheme initiated with BSE-infected cattle brain homogenate as the spike
- Aliquots removed from spiked heparin and the four purification steps
  - Assay infectivity by animal bioassay
  - RT-QuIC in vitro assay to detect PrP^{TSE}
- Bioassay with transgenic mice expressing the bovine prion protein

BSE study update

- Mouse bioassays are ongoing (completion expected at the end of next year)
- RT-QuIC assay with BSE cattle brain homogenate and heparin required modifications
- RT-QuIC studies are ongoing

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