

Prion-like Proteins: Implementation of IBC Oversight

Susan E. Vleck, PhD, RBP

Senior Biosafety & Biosecurity Specialist
Manager, Animal Research Occupational Health & Safety
Environmental Health & Safety
Stanford University

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Prion Diseases

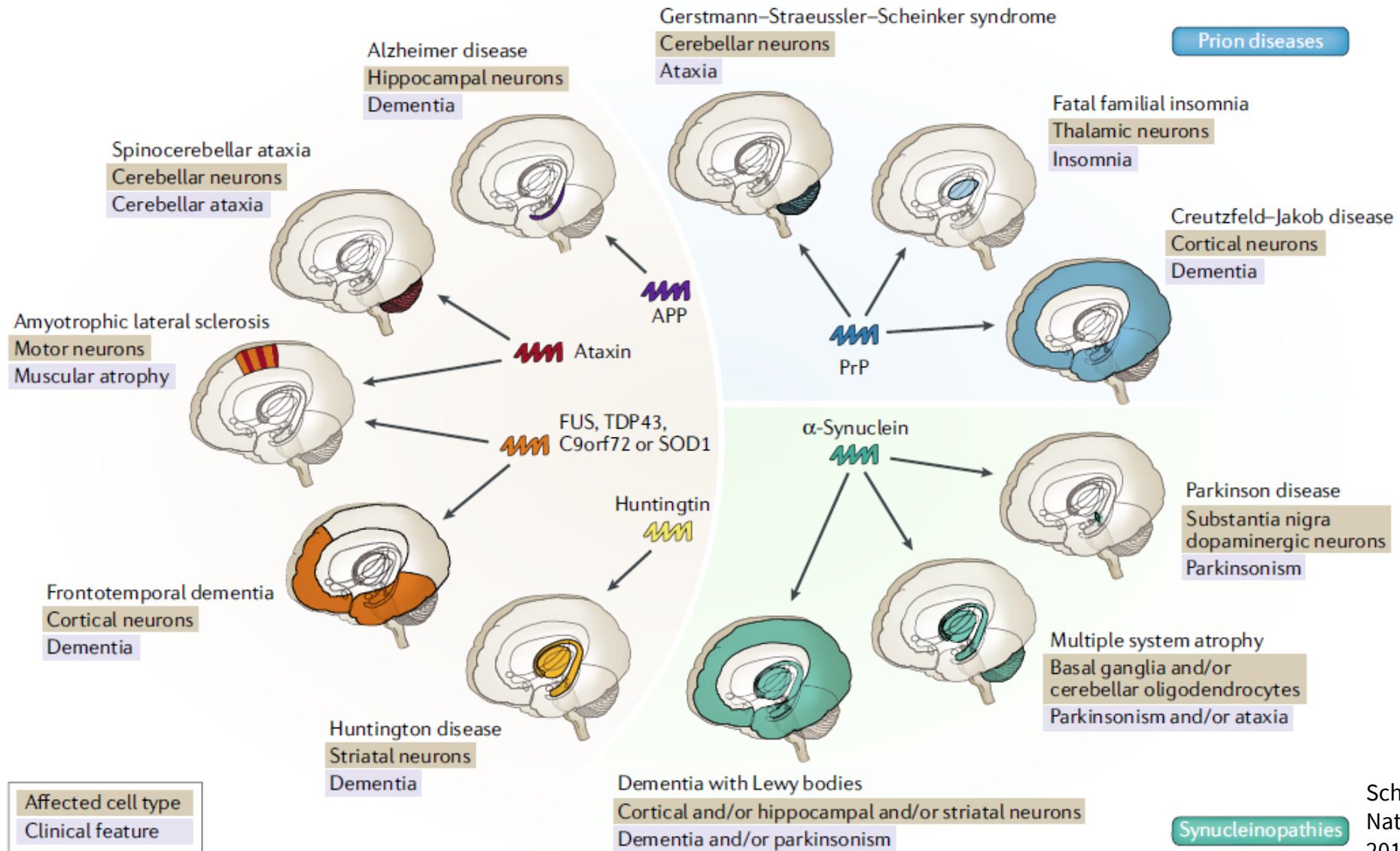
- Transmissible Spongiform Encephalopathy (TSE)
 - Rare, progressive, always fatal
- Affect both humans and animals
- Transmission
 - Genetic inheritance
 - Ingestion/exposure to infected tissues/homogenates
 - Cross-species transmission
- Normal cellular protein form misfolds into disease-associated protein form
 - “Seeds” further misfolding (akin to crystal growth)



Humans	Animals
Creutzfeldt-Jakob disease (CJD)	Bovine SE (Mad Cow)
Kuru	Chronic Wasting disease(deer/elk)
Fatal Familial Insomnia	Scrapie (sheep)
Gerstmann-Straussler-Scheinker Syndrome	Transmissible mink SE
	Feline SE
	Ungulate SE

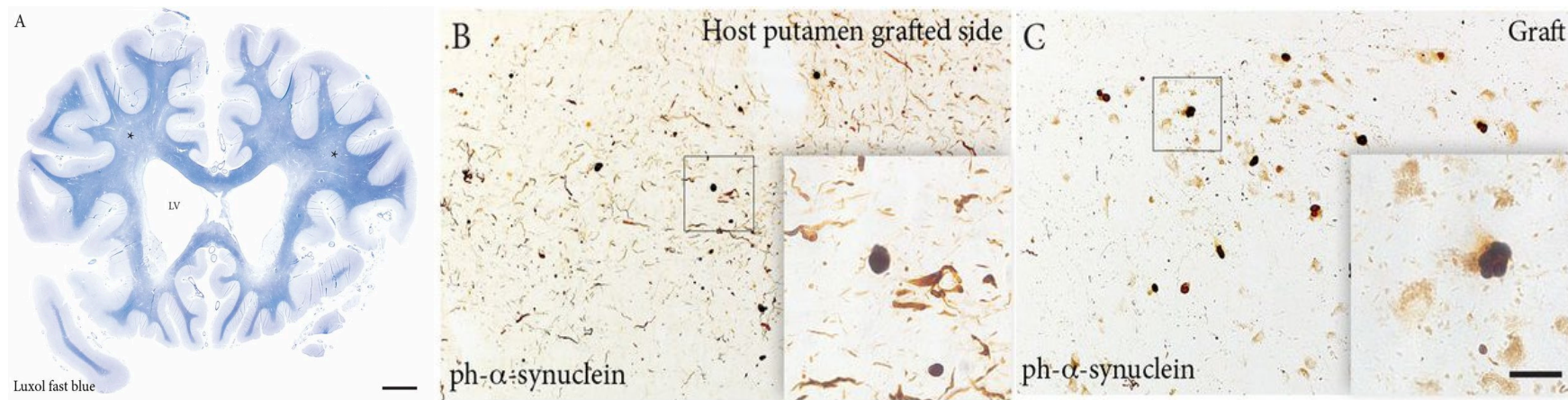
Other disease-associated proteins that seed/aggregate?

Box 2 | Protein misfolding disorders affecting the nervous system



Risk Assessment: Evidence for prion-like properties

Seeding	Brain homogenates or purified proteins transmit pathology in mice
Cell-to-cell transmission	Neuronal transplants show pathology within the grafted areas (host-to-graft transmission)
Cross-species transmission	Human aggregates transmit pathology in mice
Resistance to degradation	Formalin fixed samples still transmit pathology in mice
Human-to-human transmission	No evidence <ul style="list-style-type: none"> • Not a public health issue! • What about a researcher health issue?



Li et al., PNAS, 2016

Biosafety Level

- BMBL: BSL2 practices/procedures
 - Stanford IBC: Work with known mutations or fibrillary/aggregated protein done at BSL2
- WHO/CDC: patient care
 - Special considerations for equipment and PPE in surgical procedures, autopsies, etc.
- Standard BSL2 + Clinical = Prion-like Practices
 - Special considerations
 - Decontamination/disposal
 - Fixation
 - Post-exposure management



WHO/CDS/CSR/APH/2000.3

WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies

Report of a WHO consultation
Geneva, Switzerland, 23-26 March 1999

World Health Organization
Communicable Disease Surveillance and Control

This document has been downloaded from the WHO/EMC Web site. The original cover pages and lists of participants are not included. See <http://www.who.int/emc> for more information.

Prions: Disinfection is a unique challenge

Table 8 Ineffective or sub-optimal disinfectants

Chemical disinfectants	Gaseous disinfectants	Physical processes
<u>Ineffective</u> ¹⁷ alcohol ammonia β-propiolactone formalin hydrochloric acid hydrogen peroxide peracetic acid phenolics sodium dodecyl sulfate (SDS) (5%)	<u>Ineffective</u> ethylene oxide formaldehyde	<u>Ineffective</u> boiling dry heat (<300°C) ionising, UV or microwave radiation
<u>Variably or partially effective</u> chlorine dioxide glutaraldehyde guanidinium thiocyanate (4 M) iodophores sodium dichloro-isocyanurate sodium metaperiodate urea (6 M)		<u>Variably or partially effective</u> autoclaving at 121°C for 15 minutes boiling in 3% sodium dodecyl sulfate (SDS)

Effective Methods

- Incineration
 - Above 1000°C
- NaOH
 - +/- autoclave
- Sodium hypochlorite
 - 20,000ppm (40% v/v)
 - +/- autoclave

PPE

- Standard BSL2:
 - **Sufficient!**
 - Lab coat, gloves, eye protection as needed
 - Kevlar gloves under latex/nitrile gloves
 - Cryotome blades, scalpels (not sufficient against needle sticks)
- Clinical: No additions
- Safety concerns:
 - Getting researchers to wear PPE



Safety Goggles



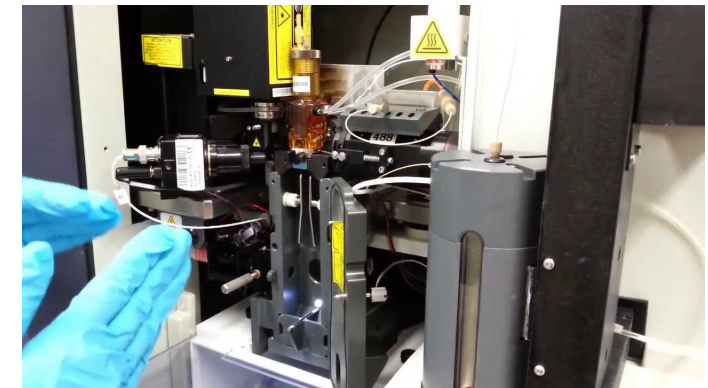
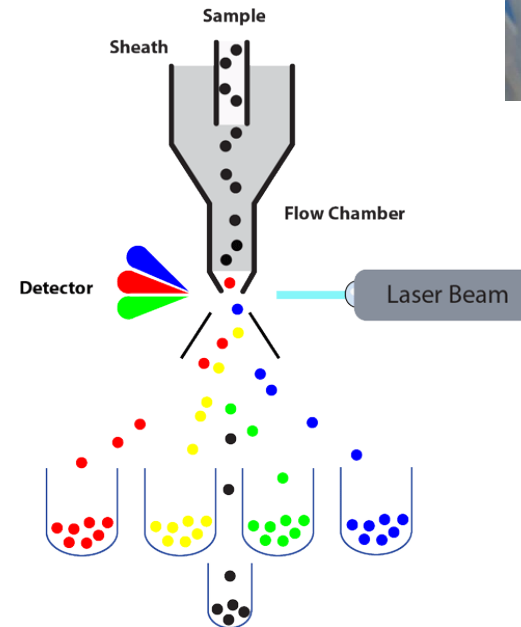
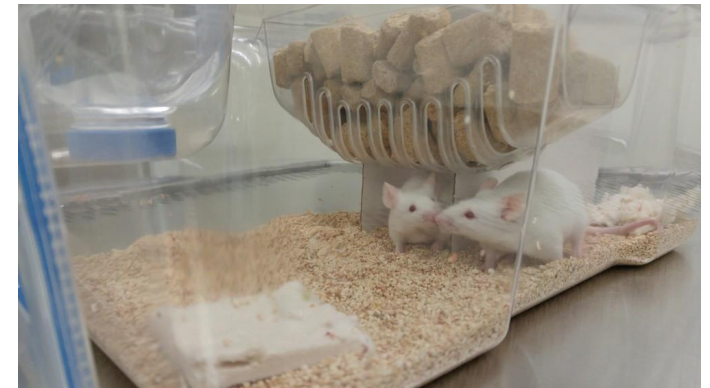
Labcoat



Gloves

Equipment and supplies

- Standard BSL2:
 - BSC, aerosol & sharps precautions, etc.
- Clinical: **ADD**
 - Utilize disposable equipment wherever possible
 - Dedicate permanent equipment to the work
- Safety concerns:
 - Shared equipment
 - Delicate equipment



Animal Work



- Standard BSL2:
 - ABSL2: PPE, housing, training, signage
 - Direct administration of proteins or homogenates
 - “Shedding” period, allowed move to ABSL1+
 - ABSL1+: normal housing, updated cage cards
 - Transgenic or naturally occurring models
- Clinical: **Already in place**
 - All animal biohazard waste incinerated
- Safety concerns:
 - Implanted pumps
 - Sharps for inoculation

Lab Changes cage

First cage change done by the lab: ___/___/___

BIOHAZARD

Barcode: 121212 Phone #: emergency phone here
PI: PI name here
Responsible Person: RP name here
Email: RP@stanford.edu
Agent: E. coli Infection date: 01/01/2017
ABSL1+ ABSL2 (check one)

VSC Changes Cages

First cage change done by the lab: 01/01/17

BIOHAZARD

Barcode: 121212 Phone #: emergency phone here
PI: PI name here
Responsible Person: RP name here
Email: RP@stanford.edu
Agent: AAV Infection date: 01/01/2017
ABSL1+ ABSL2 (check one)

Fixation

- Standard BSL2:
 - PFA, Glutaraldehyde, etc., **insufficient**
- Clinical: **UPDATE**
 - 95-100% formic acid for 1 hour
 - Fresh 10% formalin for 48 hours
 - Gentle agitation
- Researcher concerns:
 - Maintaining delicate epitopes for antibody binding
- Adaptation:
 - Allow standard BSL2 fixation
 - Continue to handle at BSL2



Work Area and Surfaces

- Standard BSL2:
 - Utilize BSL2 facilities
 - Common 10% bleach **insufficient**
- Clinical: **UPDATE**
 - Cover work surfaces with disposable material
 - Decontaminate surfaces with appropriate disinfectant
 - NaOH, Sodium hypochlorite
 - Water wipe to remove residue
 - Flood surfaces for 1 hour following spill
- Safety concerns:
 - Chemical hazards
 - Appropriate waste stream



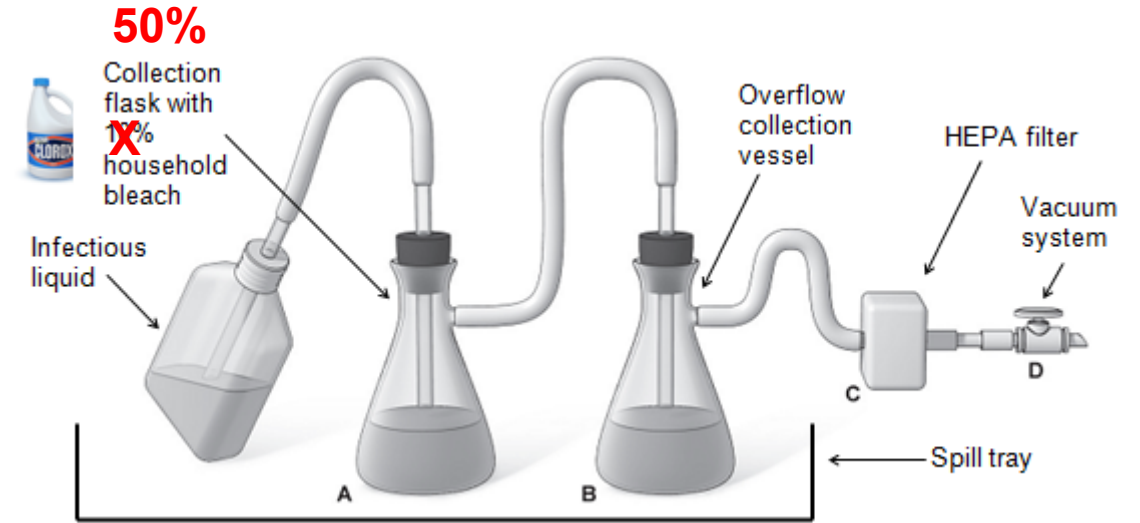
Solid Waste and Sharps

- Standard BSL2:
 - Common autoclaving or biohazard waste **insufficient**
- Clinical: **UPDATE**
 - Incinerate
 - Soak in NaOH/sodium hypochlorite and autoclave
 - Autoclave
- Safety concerns:
 - Documentation
 - Autoclave ability (and resetting)
- Adaptation:
 - Chemo/bio bins or Pathology bins
 - Perk: mixed solid and sharps waste
 - Incinerated by medical waste handler (verified temp)



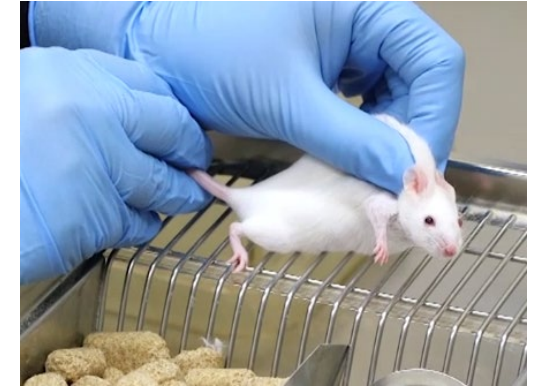
Liquid Waste

- Standard BSL2:
 - 10% final v/v bleach **insufficient**
- Clinical: **UPDATE**
 - NaOH or Sodium hypochlorite
- Safety concerns:
 - Chemical hazard
- Adaptation:
 - 50% final v/v household bleach is easy math
 - Dedicated tubing that is eventually disposed
 - Waste collected as chemical waste
 - Education of pickup crew!



Occupational Health: Individual exposure

- Standard BSL2:
 - Soap and water for 5-15 minutes
 - Encourage bleeding for needle sticks/lacerations
 - Irrigate with water for 15 minutes
 - Further medical treatment as needed
- Clinical: **ADD**
 - Brief exposure, 1 minute, to 0.1 N NaOH or 1:10 dilution of bleach
- Safety concerns:
 - Wrong dilution of NaOH/bleach used
 - Provide exposure response kit?



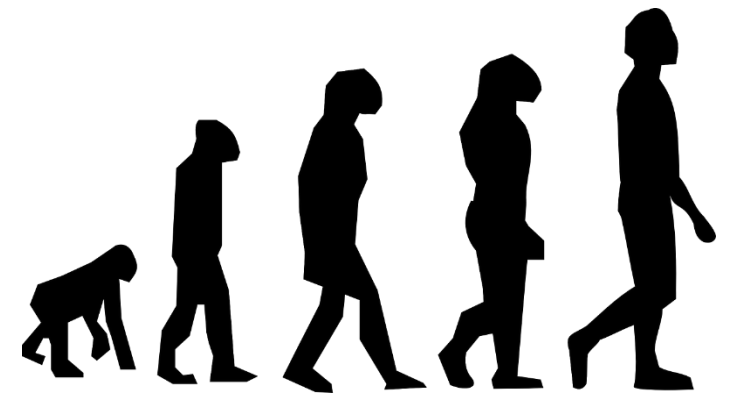
Occupational Health: Public Health

- NIH is preparing a White Paper
 - Alzheimer's, Parkinson's, etc., are NOT transmissible
 - No extra clinical precautions for work with patients
- No evidence of human-to-human transmission
 - No public health threat
- Does not negate the research exposure risk
 - Needle stick with fibrillary protein? Homogenate?
 - Unknown potential outcome



Biggest consideration: Pushback!

- Be prepared
 - Have your IBC Chair on your side
 - Talk to your Neuro, Pathology chairs
- Understand that not all researchers agree with the science
 - True of all scientific fields—don't take it personally, but stand your ground
 - Or, stand your Chair's ground
- Stress the guideline as a living document
 - Can change as the science updates
- Acknowledge the need to adapt
 - Make practical decisions regarding implementation



Questions?

Susan E. Vleck, PhD, RBP
Senior Biosafety & Biosecurity Specialist
Manager, Animal Research Occupational Health & Safety Program
Stanford University

sevleck@stanford.edu

650-724-7818

<http://ehs.stanford.edu>

Thank you!