

**Laboratory of Bacterial, Parasitic
and Unconventional Agents (LBPUA)
Research Programs**

Report to BPAC 13 July 2006

**Site Visit by Ad Hoc Review Committee
25 May 2006**

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**Division of Emerging and Transfusion-
Transmitted Diseases**

**Office of Blood Research and Review
Center for Biologics Evaluation and Research
United States Food and Drug Administration**

Goals of LBPUA Research Programs

- **Product development and safety**
 - *Leishmania*, Chagas' disease, malaria, TSEs: candidate blood donor tests
 - *Leishmania*, malaria vaccines; TSEs, vaccine safety
- **Other regulatory-relevant research**

- Pathogenesis at cellular and molecular levels
Leishmania, malaria, TSEs

Justification for research on pathogenesis

- Provides materials and information of regulatory relevance
- Improves reviewer insight into regulatory issues
- Maintains scientific expertise, commitment to mission
- Usually self-supporting (supplements other funding)

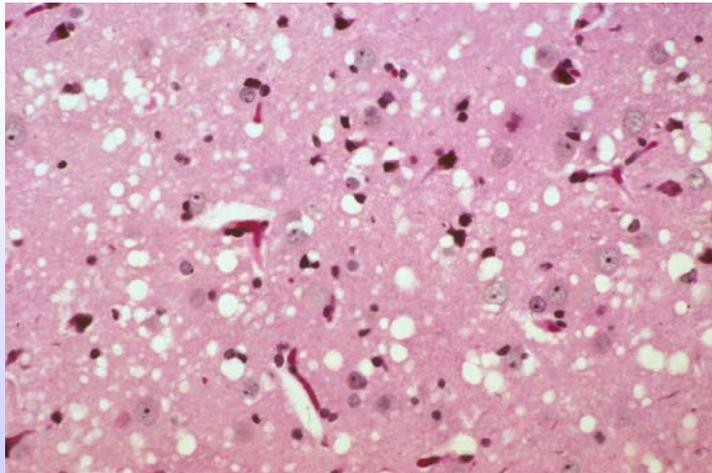
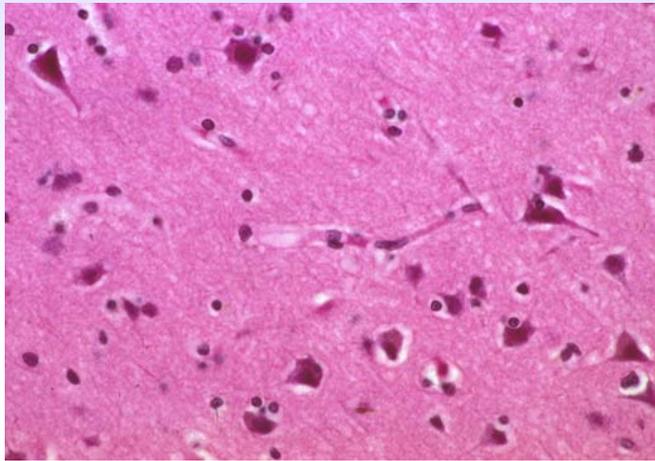
Two LBPUA Research Programs (5 PIs)

- **Parasitic and bacterial diseases**
 - ***Leishmania* vaccine safety, blood donor screening assays [3 staff incl PI, R Duncan, A Selvapandyan]
PI: HL Nakhasi, PhD (Director, DETTD)**
 - ***Leishmania, T. cruzi* pathogenesis, role in blood safety [3 staff incl PI]
PI: A Debrabant, PhD**
 - **Malaria pathogenesis, role in vaccine safety, role in blood safety, blood donor screening assays (transfusion-transmitted bacterial infections) [4 staff incl PI]
PI: S Kumar, PhD**
- **Transmissible spongiform encephalopathies**
 - **Mfg safety, blood donor screening assays* [5 staff incl PI]
PI: D Asher, MD (Chief, LBPUA)**
 - **Pathogenesis, source materials safety [3 staff incl PI]
PI: P Piccardo, MD**

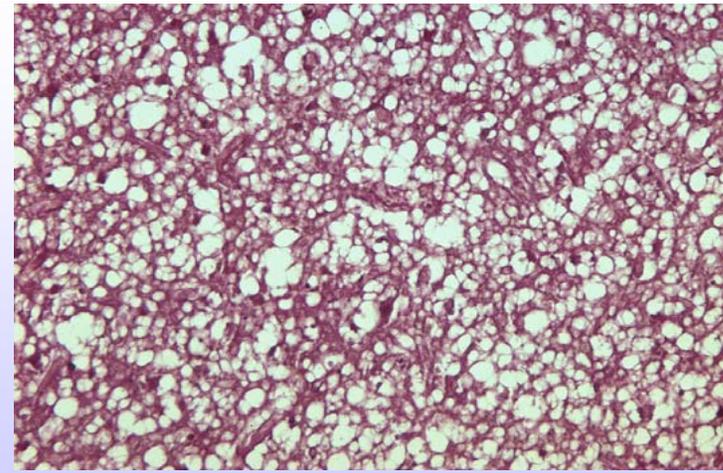
* cf. WHO Consultation September 2005: <http://who.int/bloodproducts/tse/WHO%20TSE%20Guidelines%20FINAL-22%20JuneupdatedNL.pdf>

Transmissible Spongiform Encephalopathies

normal brain



spongiform degeneration



status spongiosus

LBP UA TSE Research Program: 2 Sections

- **TSE Pathogenesis and Source Materials Safety**
(PI: P. Piccardo)

Maintain human-derived/animal-derived materials TSE-free

- **TSE Manufacturing Safety Section**
(D. Asher)

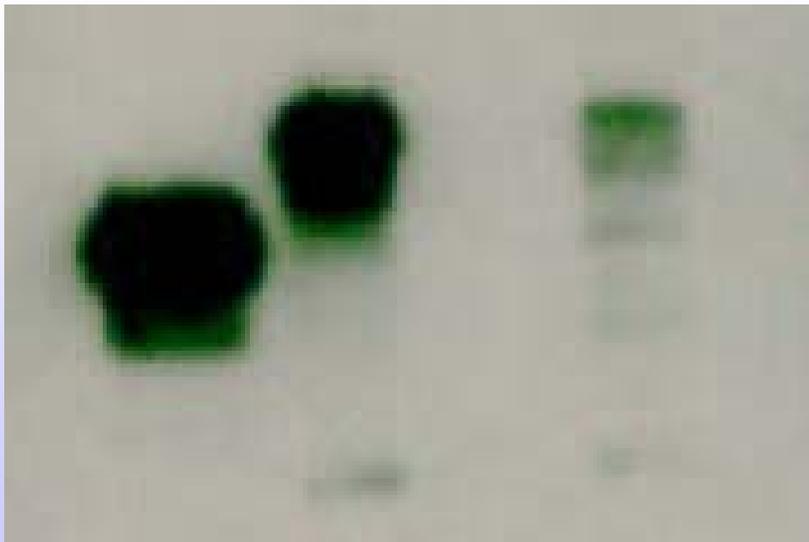
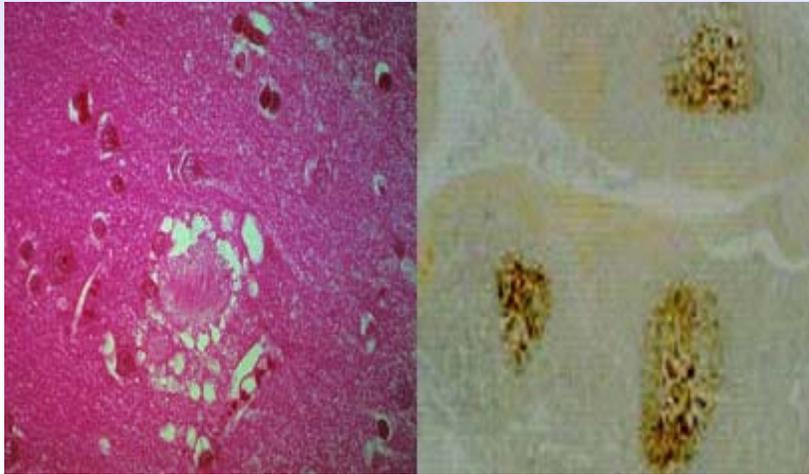
Keep manufacturing facilities/equipment TSE-free

- 1. Evaluate cleanup/disinfection methods for TSEs**
- 2. Develop computerized morphometric analysis (CMA) of objective, quantifiable immunohistochemical criteria to study/diagnose TSEs: support tissue/surgical safety**
- 3. Evaluate susceptibility of biologics cell substrates to TSE infections (NIAID-FDA Inter-Agency Agreement)^a**
- 4. Establish US TSE Biological Reference Materials: human (and ?animal) TSE brain, other tissues, blood components^{a,b}**

^aJoint efforts of both LBP UA TSE Sections ^{a,b} Proposed project

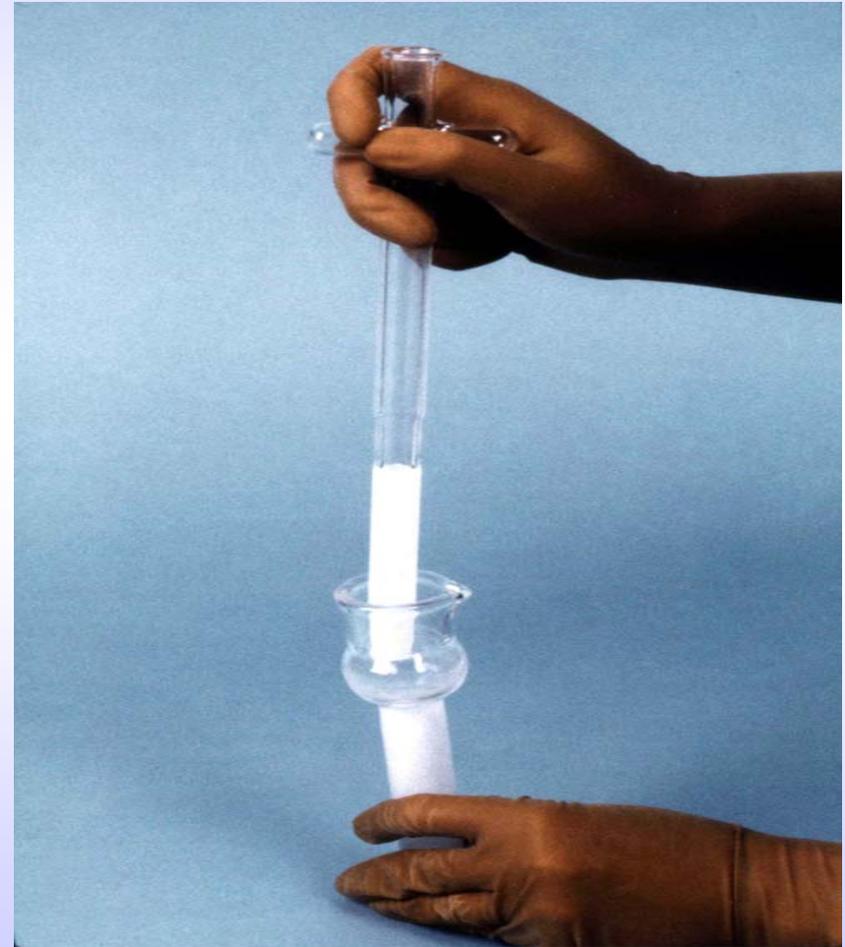
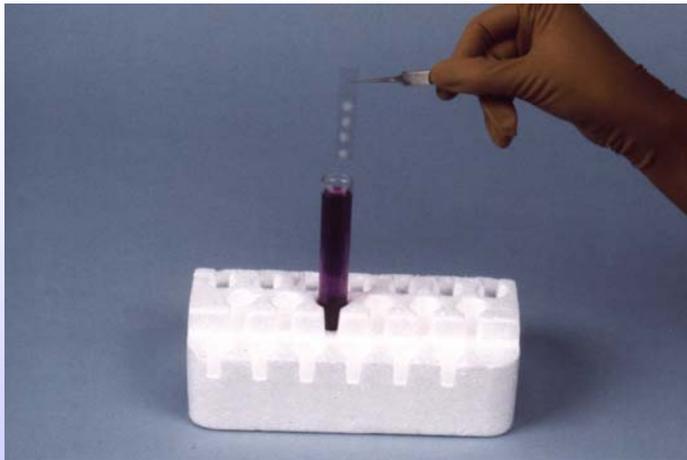
Abnormal prion protein (designated PrP^{Sc}, PrP^{res} or **PrP^{TSE}**)

- derived from ubiquitous normal precursor protein (PrP^C)
- useful marker of TSE infections (IHC, WB, ELISAs)



- PrP^{TSE} is an N-truncated abnormally folded cleavage product (high β -sheet) of normal “cellular” protein (PrP^C),
- relatively insoluble in detergent-salt solutions, and
- relatively resistant to digestion with proteinase K (PK).
- PrP^C is a 253-aminoacid peptide encoded by the *PRNP* gene on human chromosome 20.
- Familial TSEs are linked to a number of *PRNP* point mutations, deletions or inserted duplications.
- Mice lacking PrP-encoding genes cannot be infected with TSE agents.
- PrP^{TSE} alone ?? = infectious TSE agent

Adaptation of Chen-Koski glass crush method for evaluation of virucides to study TSE agents



Adaptation of Zobeley-Flechsigg-Weissmann steel wire method to evaluate TSE decontamination



Titrations of 263K scrapie agent dried on glass slips or steel pins: ultrasonic wash in hot alkaline detergent

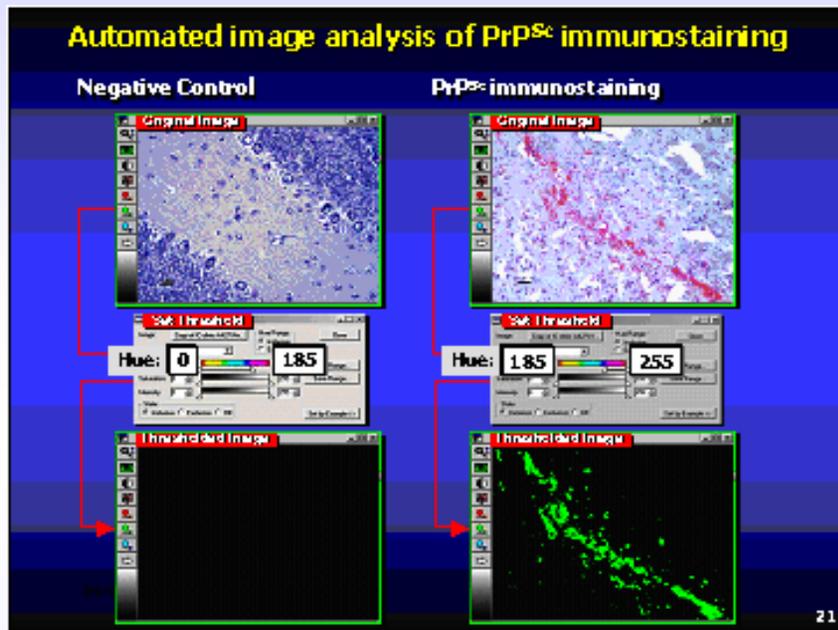


	Temp x Time	Log₁₀ LD₅₀ Reduction (Residual Infectivity)
Glass Slip	61°C x 90'	5.3 (2.7)
Steel Pin	65°C x 90'	5.6 (1.2)

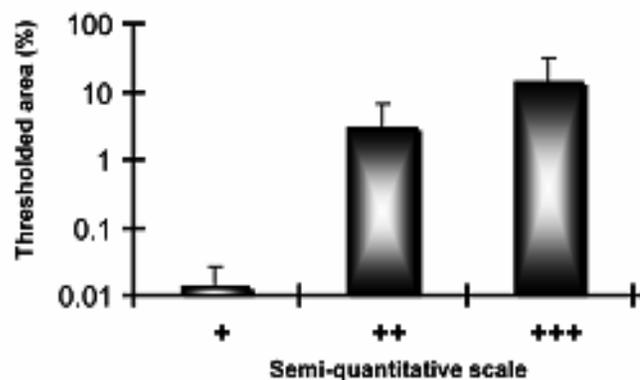
**263K scrapie agent dried on steel pins (~6 log₁₀ hamster IC LD₅₀) “WHO” decontamination methods
plus ultrasonic cleaning in hot alkaline detergent &c**

Decontamination regimen	scrapie-infected pins/ total pins tested
Controls (normal brain)	0/90
NaOCl 6% 22°C 60’	0/36
NaOH 2N 22°C 60’	0/36
NaOH 2N 100°C 20’	0/37
NaOH 2N SDS 3% 134°C 18’	0/32
Proprietary phenolic (as directed) 22°C 60’	0/35
H₂O 134°C 18’	0/39

Computerized morphometric analysis (CMA) of immunostained prion protein in scrapie hamster brains correlates well with conventional scores.



- Original images (top) transformed to CMA-thresholded (i.e., positively stained) images. Controls on left, scrapie on right



- Good correlation between conventional visual scores (0, +, ++, +++) and CMA values expressed as % of area stained for replicate images

Susceptibility of biologics cell culture substrates to TSE infections

1. Artificial worst case

Expose engineered human neuronal-glial cell lines overexpressing wild-type or mutant PrP to TSE agents:

30 serial passages, then bioassay for infectivity, various assays for PrP^{TSE}

2. Actual cell substrates

Expose proposed cell substrates to TSE agents:

30 serial passages, then bioassay, PrP^{TSE} assay

- Vero (green monkey kidney heteroploid cells)
- CHO (Chinese hamster ovary heteroploid cells)
- HEK 293 (human fetal kidney cells transformed with defective-adenovirus)
- WI-38 (? MRC-5) human diploid fibroblast cells
- Other as indicated by actual biologics development (MDCK)

**Experiments inoculating various cell lines with TSE agents,
serial passaging (√ = passage completed, cells stored)**

Cell line	sCJD Passages						BSE Passages					
	0	5	10	15	20	30	0	5	10	15	20	30
SH-SY5Y	√	√	√	√	√	√	√	√	√	√		
SH-SY5Y-wt	√	√	√	√	√	√	√	√	√	√		
SH-SY5Y-E200K	√	√	√	√	√	√	√	√	√	√		
CHO	√	√	√	√	√	√	√	√	√	nd	√	√
Vero	√	√	√	√	√	√	√	√	√	nd	√	√
WI38 (later MRC-5)			planned				√	√	√	√	√	√
MDCK			planned				√	√	√	√	√	√
HEK 293			planned						planned			
R9ab	√	√	√	√	√	√	√	√	√	√	√	√
Mo3F4			not planned						planned			
3T3			not planned						planned			

Proposed TSE Blood-derived Biological Reference Reagents

Species	Possible TSE agents
Rodent (mouse, hamster)	Scrapie, BSE, sCJD, vCJD
Sheep	Scrapie, ?BSE
Monkey	vCJD, ?sCJD, ?BSE
Chimpanzee	GSS (available), ?? other
Human	sCJD, fCJD (antemortem) vCJD, other (postmortem)