

Division of Emerging and Transfusion Transmitted Diseases (DETTD)

**Hira Nakhasi, Ph.D.
Director**

OBRR Office Site Visit July 22, 2005

Organization of the Division of Emerging and Transfusion Transmitted Diseases (DETTD)

CBER-OBRR
DIVISION OF
EMERGING &
TRANSFUSION
TRANSMITTED
DISEASES

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*Laboratory of Hepatitis & Related
Emerging Agents*
Gerardo Kaplan, Ph.D.

Division of Emerging and Transfusion Transmitted Diseases (DETTD) Mission

- ◆ **Proactively ensuring the safety of the blood supply through:**
 - ✦ **Regulation of blood screening and diagnostic tests to detect infectious contaminants of blood products**
 - ✦ **Evaluation of new technologies for rapid and flexible screening of the blood supply**
 - ✦ **Development and revision of FDA Guidance for users of blood screening and diagnostic products**
- ◆ **Plan and conduct research**
 - **Disease pathogenesis, safety of tests for blood-borne pathogens and development of biomarkers for vaccine safety of blood-borne agents**

Division of Emerging and Transfusion Transmitted Diseases (DETTD) Mission continued...

- ◆ **Lot release testing:**
 - for approval of investigational tests and surveillance of licensed products
 - develops reference materials for lot release testing
- ◆ **Inspections:**
 - of manufacturers of licensed products, manufacturing facilities
- ◆ **Consultation:**
 - provide expert scientific and technical advice to other Agency and Government components
- ◆ **Outreach:**
 - safety and efficacy of blood donor screening testing at the Blood Product Advisory Committee, TSE Advisory Committee, DHHS Blood Safety Availability Committee

Regulatory Output for FY'04

- **BLAs**
 - ✓ Original = 1
 - ✓ Supplements = 45
 - ✓ Amendments = 114
 - ✓ Annual Reports = 28
- **PMAs/510(K)s = 60**
- **IND/IDEs**
 - ✓ Originals = 19
 - ✓ Amendments = 311
- **Thousands** of test kit lots tested and released
- Performed **8** inspections and several laboratory investigations

Research Output for FY '04

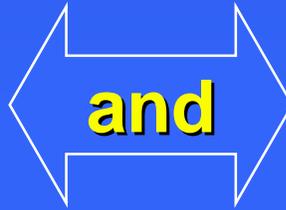
◆ 9 Principal Investigators

◆ Research Publications in DETTD FY '04

41

The Critical Path Challenge for Blood Products

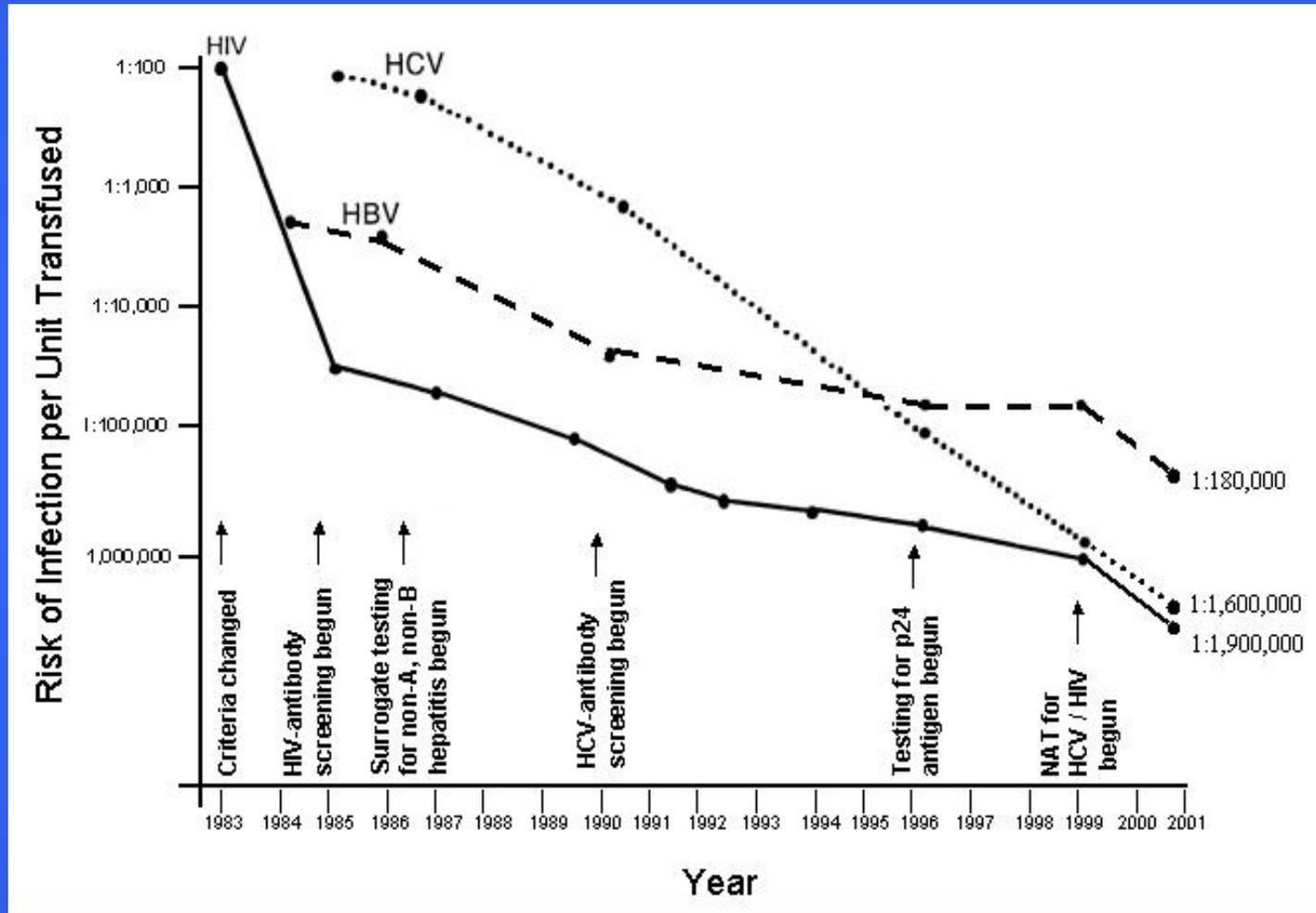
**Enhance
Product Safety,
Purity and
Potency**



**Avoid Product
Shortages & Major
Increased Costs**

Critical Path opportunities exist that could improve blood product safety, efficacy and availability while minimizing disruptions to the blood system

The Impact of Testing for HIV, HBV and HCV on the Safety of the US Blood Supply



Testing has significantly reduced the risk of transmission

DETTD Research Priorities

- ◆ **Blood Safety and Availability**
 - retroviral, hepatitis, WNV, parasitic, TSE and emerging agents (SARS, vaccinia, SENV, etc.)
- ◆ **Vaccine Safety**
 - blood-borne parasitic agents
 - HIV and hepatitis agents
- ◆ **Manufacturing**
 - CJD/vCJD detection and decontamination
 - CMC issues with screening assays

A. Blood Safety and Availability: **Impact on the US Public Health**

- **Millions of units are transfused annually**
- **Risk of transmission through transfusion has significantly reduced with the introduction of tests**

A. Blood Safety and Availability: **Impact on the US Public Health (cont.)**

- **New and emerging pathogens threaten the blood safety**
 - **HIV drug resistant and recombinant variants and HBV mutants**
 - **Ability of existing tests to identify such variants**
 - **Emerging pathogens: WNV, SARS-CoV, BT agents**
 - **Existing parasitic agents (Malaria, Chagas, Leishmania)**
 - **Potential blood donors deferred for exposure to TSE agents**
 - **Significant donor loss based on exposure to parasitic agents**
 - **No screening assays are available**
 - **Reentry of uninfected donor to ensure blood availability**

A. Blood Safety and Availability:

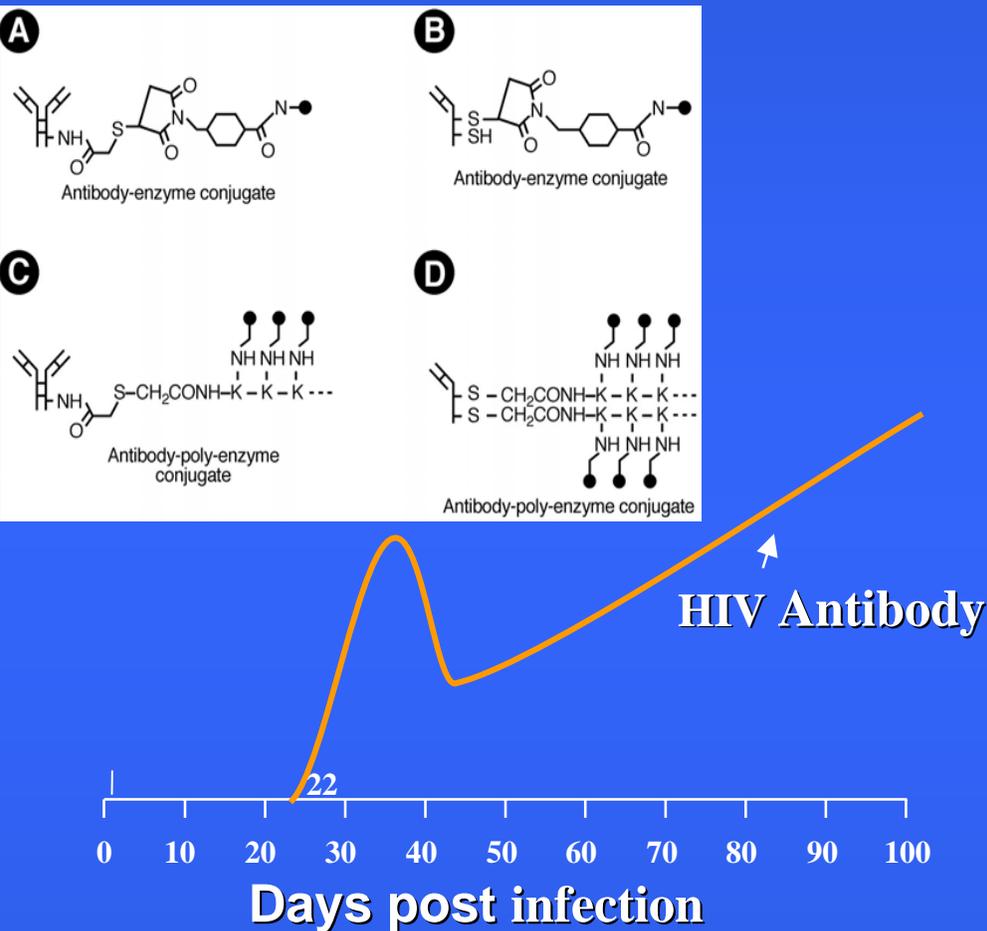
Regulatory and Scientific Challenges:

- ◆ Evaluate the sensitivity and specificity of blood donor tests for retroviruses, hepatitis, WNV, bacterial , parasitic, TSE, BT, emerging agents and diagnostic tests for retroviruses
- ◆ Maintain the safety of the blood supply by assessing the efficacy of screening and diagnostic tests in the detection of variants (drug-resistant/recombinants) of blood-borne pathogens
- ◆ Gaps in knowledge for Critical Path development of effective blood screening tests for parasites, vCJD and BT agents

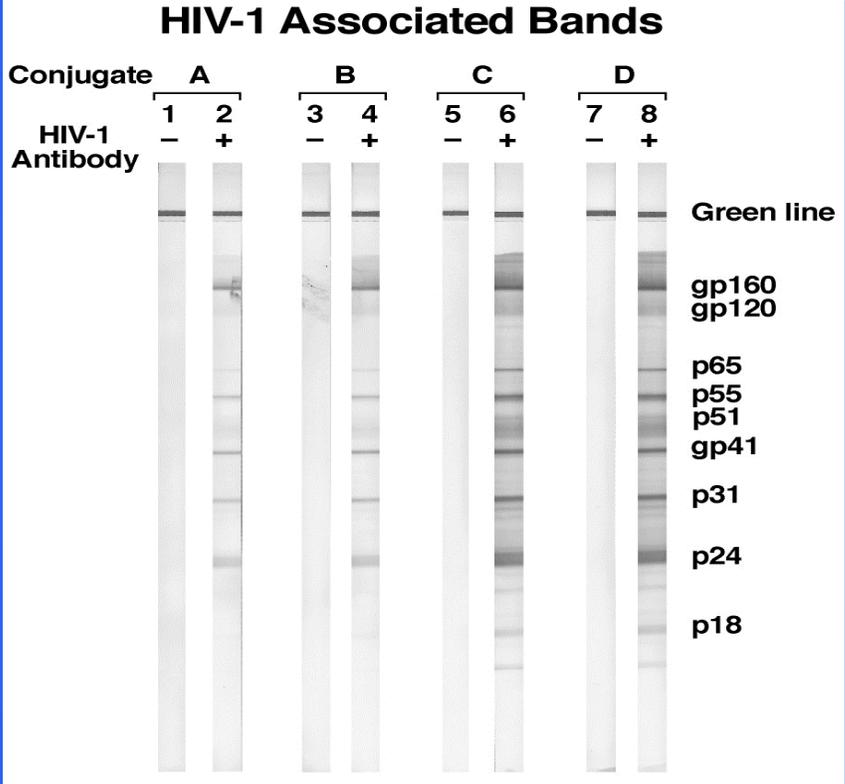
Can Currently HIV Licensed Tests Identify Retroviral Variants?

- Goal was to evaluate performance of FDA-licensed HIV assays with new variants
- CBER initiated studies in Cameroon, a country with evolving HIV diversity
- **Some CRF02_AG recombinants were not reliably detected by some NAT, IFA or EIA**
- Need for expanded national and global surveillance for emerging HIV and other human retroviral variants

Enhance the Sensitivity of Existing HIV Assays



Peptides, 23: 2091-2098 (2002)



1. Modified Ab conjugates increase sensitivity of W. blot assay
2. Such conjugates could enhance detection of Ab in early phase

Regulatory Requirements for Performance of WNV Screening Assays

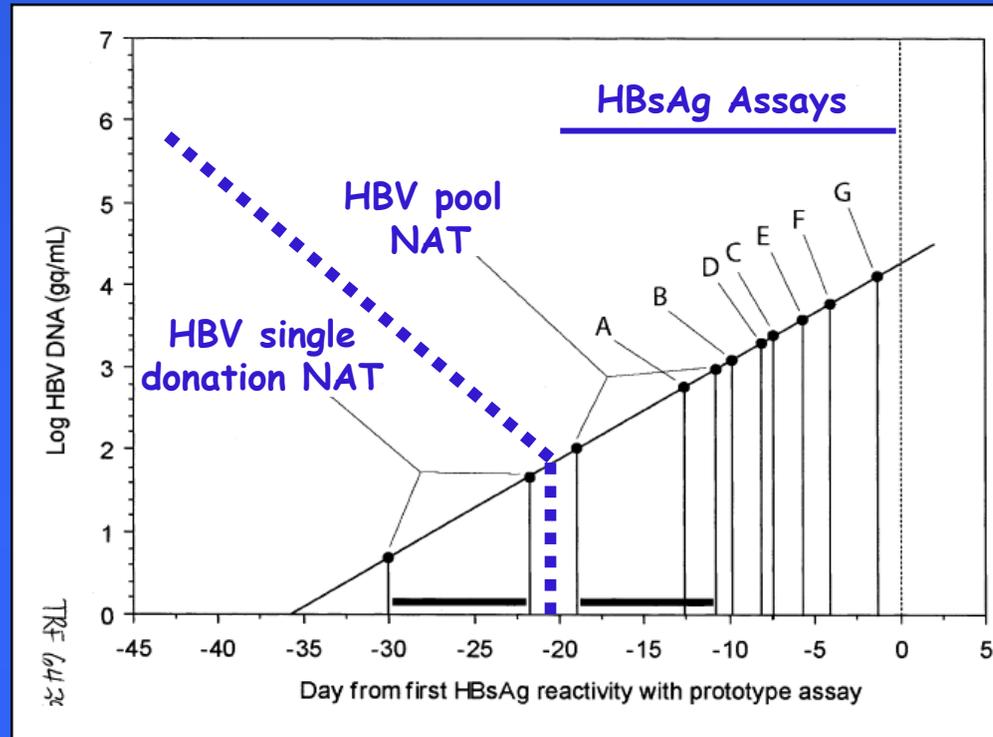
- ◆ Evaluation of Reference Panel
 - Two isolates NY99-FDA (flamingo) and FDA-Hu2002 (human)
 - Genetic characterization by sequencing
 - Biological characterization by collaborative studies
 - ✦ Correlation between infectivity titer and viral load
 - ✦ Subjected to 12 different assays performed in 7 different labs
 - Panel formulation based on collaborative studies data and reagent distribution
- ◆ FDA's current standard for WNV NAT assays for the individual donation is 100 copies/ml
 - **Most assays had satisfactory performance**

WNV Transmission by Transfusion: *In vitro* Evaluation of Protective Function of WNV-Specific Antibodies in the Presence of Low Viral Load

- Human primary cells culture system was developed
- Used to investigate infectivity of specimens potentially missed by mini-pool NAT screening containing low viral load (individual donation testing positive) and WNV-specific antibodies (MP NAT neg./ID NAT pos./Ab pos.)
- **Results showed that the presence of antibodies reduces, but does not eliminate viral infectivity *in vitro***
- Raises concern for potential risk for T-T
- *In vitro* infectivity results need to be investigated *in vivo*

Comparative Sensitivity of HBV NAT and HBsAg Assays

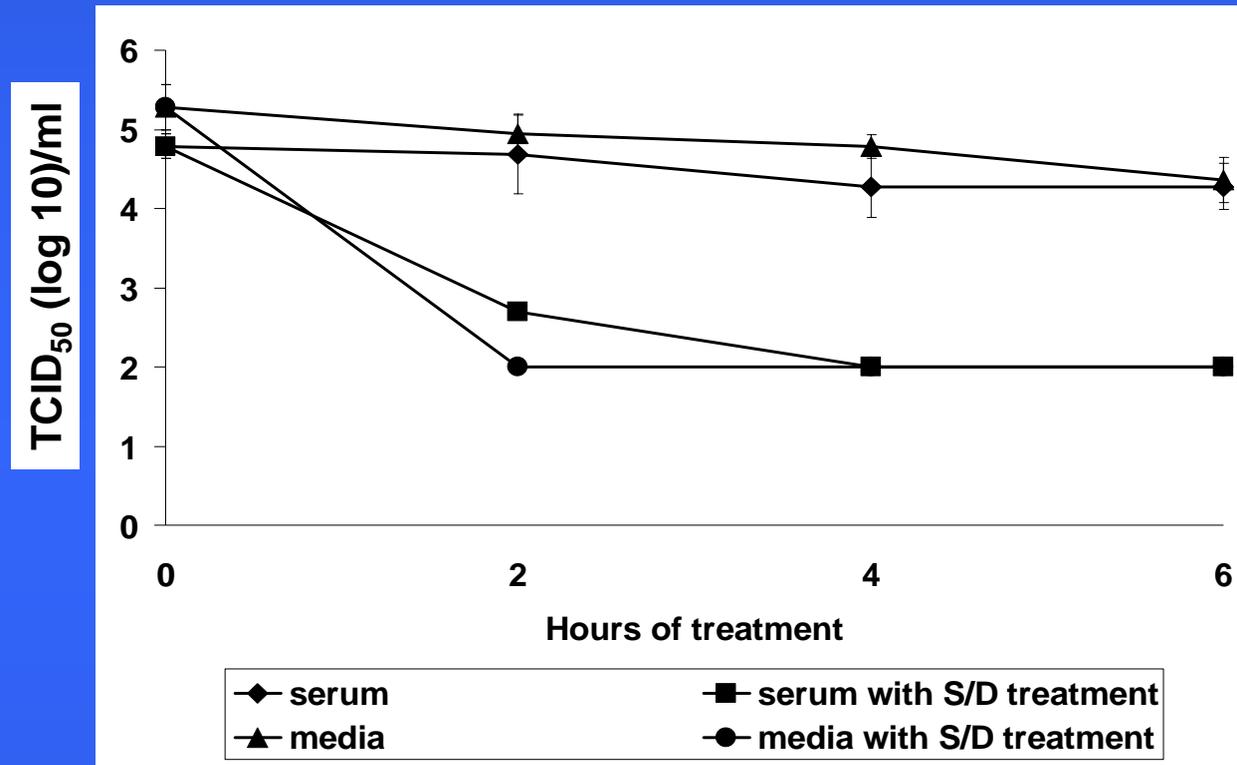
Assays for Detection of Acute HBV Infection



Biswas, et al., *Transfusion*. 2003 Jun; 43(6):788-98

- Small sensitivity increase of pooled NAT vs. HBsAg assays
- HBV pooled NAT yields a few window period cases and results in a marginal increase in safety
- **Implementation of single donation HBV NAT will most likely increase the safety of the blood supply**

Safety of Blood Products from Emerging Pathogens (SARS-Coronavirus)

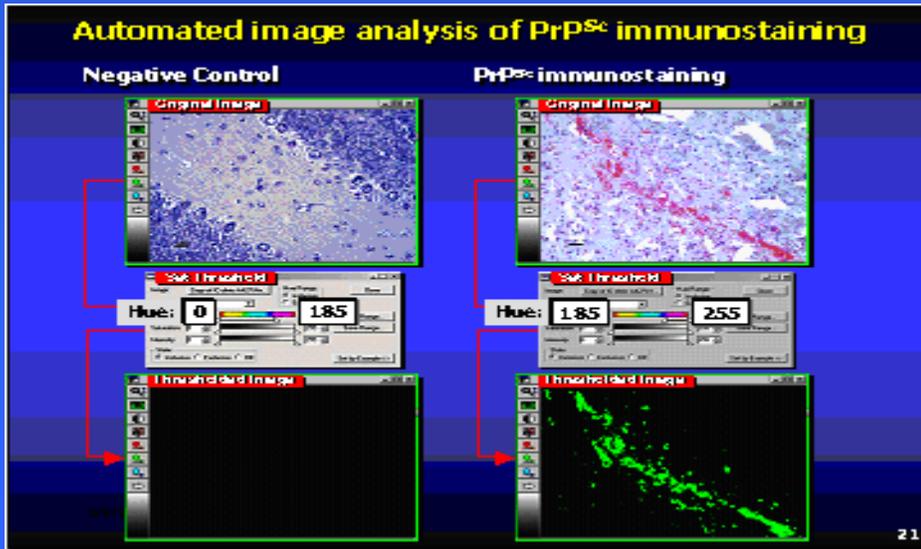


* As per New York Blood Center standard protocol

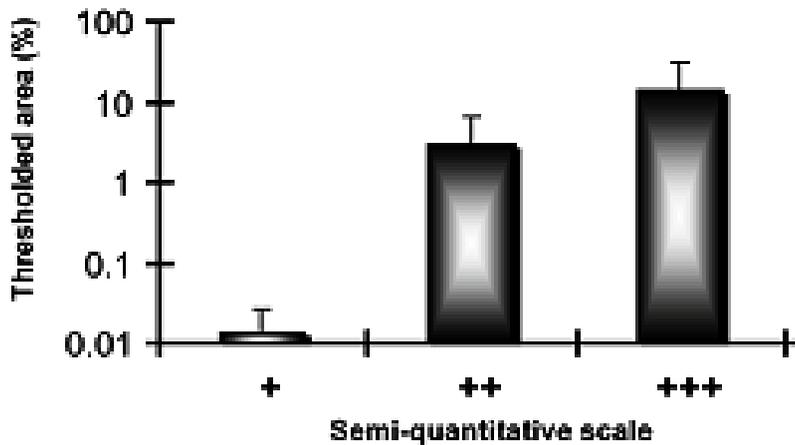
Solvent-detergent inactivates SARS-Coronavirus

- to assure safety of blood for transfusion
- blood products

Enhancing the Sensitivity of TSE Detection



- Current approach (IHC) to detect TSE is subjective
- Quantitative Morphometric Analysis (QMA) i.e. computerized digital image analysis will improve predictability and sensitivity of TSE detection



Multiplex detection of pathogens

Why is it needed?

- ◆ Increasing number of agents threaten blood safety
- ◆ Limited blood samples for detection
- ◆ Logistics of testing many agents from the same sample
 - Error rate
 - Need for rapid detection
 - Need for simultaneous detection

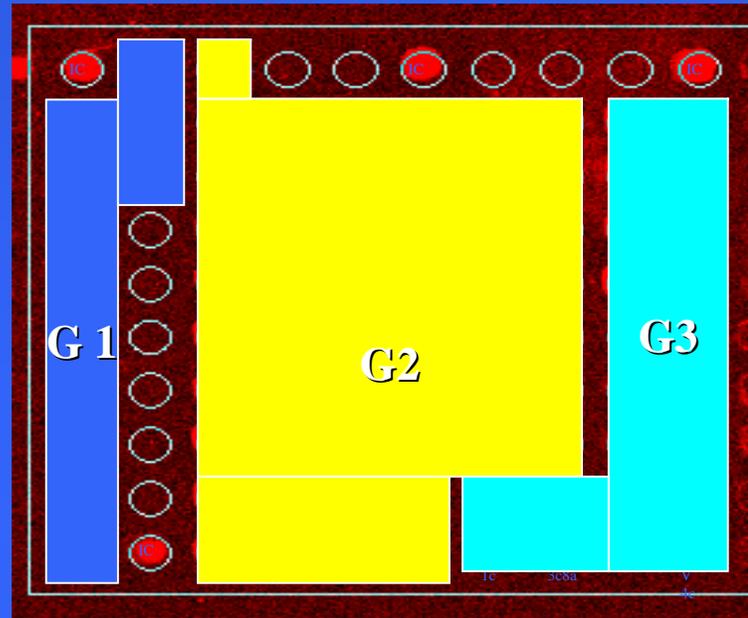
Multiplex Detection of Blood-Borne Pathogens: Oligonucleotide Microarray

Group 1: Bacteria and Parasites

Ba: *Bacillus anthracis* (**anthrax**)
 Ft: *Francisella tularensis* (**tularemia**)
 LT: *Leishmania / Trypanosoma*
 Yp: *Yersinia pestis* and *pseudotuberculosis*
 (**plague**)

Group 2: Bio-Terrorism Viruses

VAC: Vaccinia
 VAR: Variola (**smallpox**)
 MPV: Monkeypox viruses
 CPV: Cowpox viruses
 NOVAC: All Pox viruses but vaccinia
 EBO: Ebola viruses
 VE: Venezuelan Equine Encephalitis viruses
 VETD: VE Trinidad donkey
 MBG: Marburg viruses



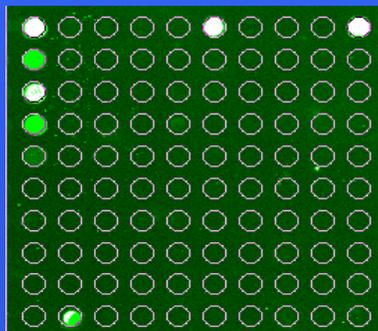
Group 3: Blood-Borne Viruses

WNV: West Nile viruses
 HCV: Hepatitis C viruses
 HBV: Hepatitis B viruses
 HIV: Human Immunodeficiency viruses
 HTLV: Human T-cell Leukemia viruses

 4 internal control probes (Human rRNA gene)

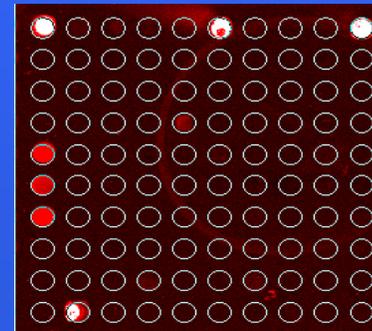
Results of detection in pathogen-spiked blood – 50 cells/ml

Bacillus anthracis



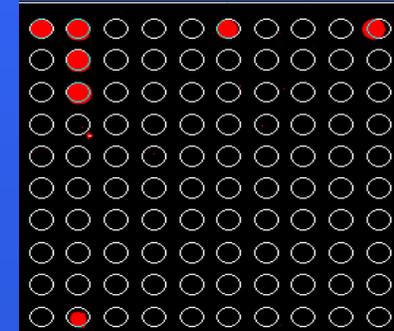
Livestock
vaccine
strain

Francisella tularensis



Live
Vaccine
Strain

Yersinia pseudotub.



B. Vaccine safety

- ◆ **Why study vaccine safety in OBRR?**
 - **CBER wide unique expertise for some of the blood borne pathogens resides in OBRR (parasitic, HAV, SARS-CoV)**
 - **Unique issues with vaccine and blood product safety**
 - ✦ **Testing for agents in vaccinees as blood donors**
 - ✦ **Role of therapeutic vaccines**
 - ✦ **Gaps in knowledge for critical path development of vaccines**

B. Vaccine Safety:

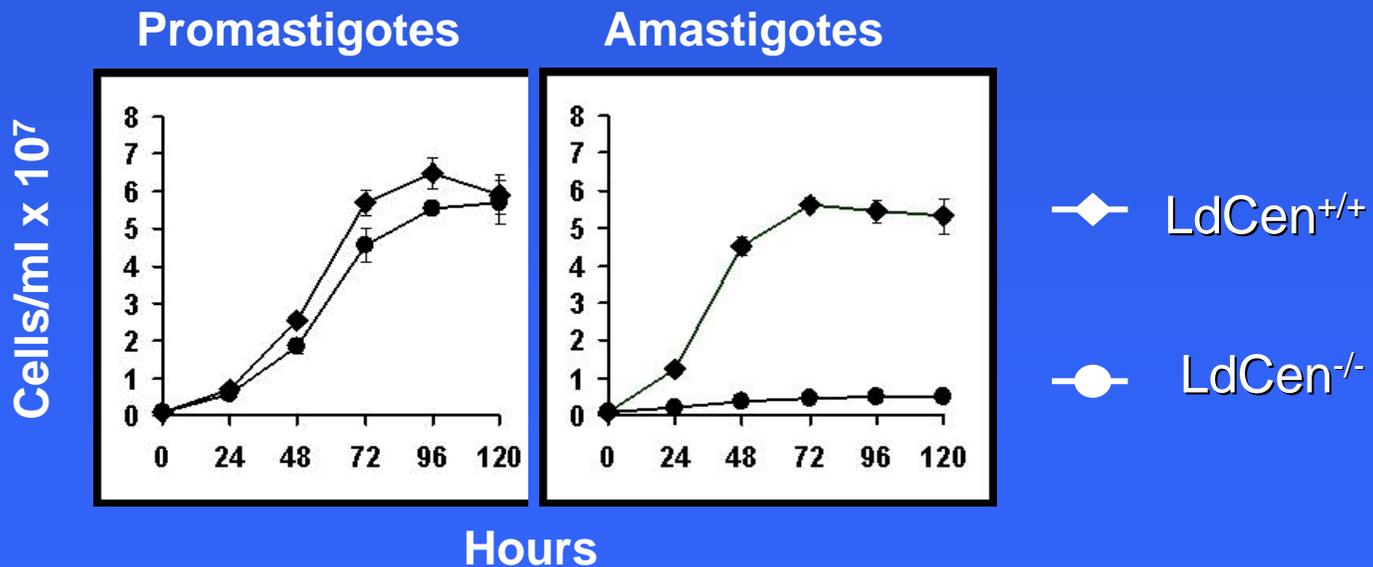
Impact on the US Public Health

- **Millions of American travelers and thousands of US troops are visitors or deployed in malaria, Chagas and Leishmania endemic areas**
 - **Increasing rates of immigration raises concern about the potential for transmission**
 - **Known cases of transmission through transfusion**
 - **Significant number of potential donors are deferred**
 - **No vaccines are available**
- **Millions of HIV and HCV infected cases worldwide**
 - **Lack of safe and effective HIV and HCV vaccines**

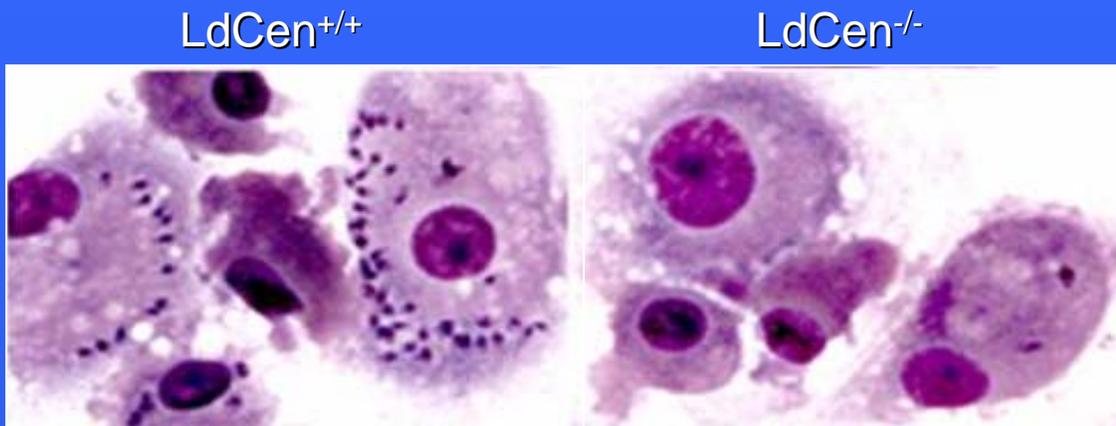
B. Vaccine Safety: **Regulatory and Scientific Challenges**

- ◆ **Assessment of safety and development of biomarkers for effective parasite vaccines**
- ◆ **Cell culture for human hepatitis viruses as an aid in diagnosis and to evaluate vaccine safety**
- ◆ **Assessment of efficacy of viral (HIV, WNV) therapeutic vaccines**
- ◆ **Selection of markers for donor screening in vaccinees**

Safety of Attenuated Leishmania Vaccine



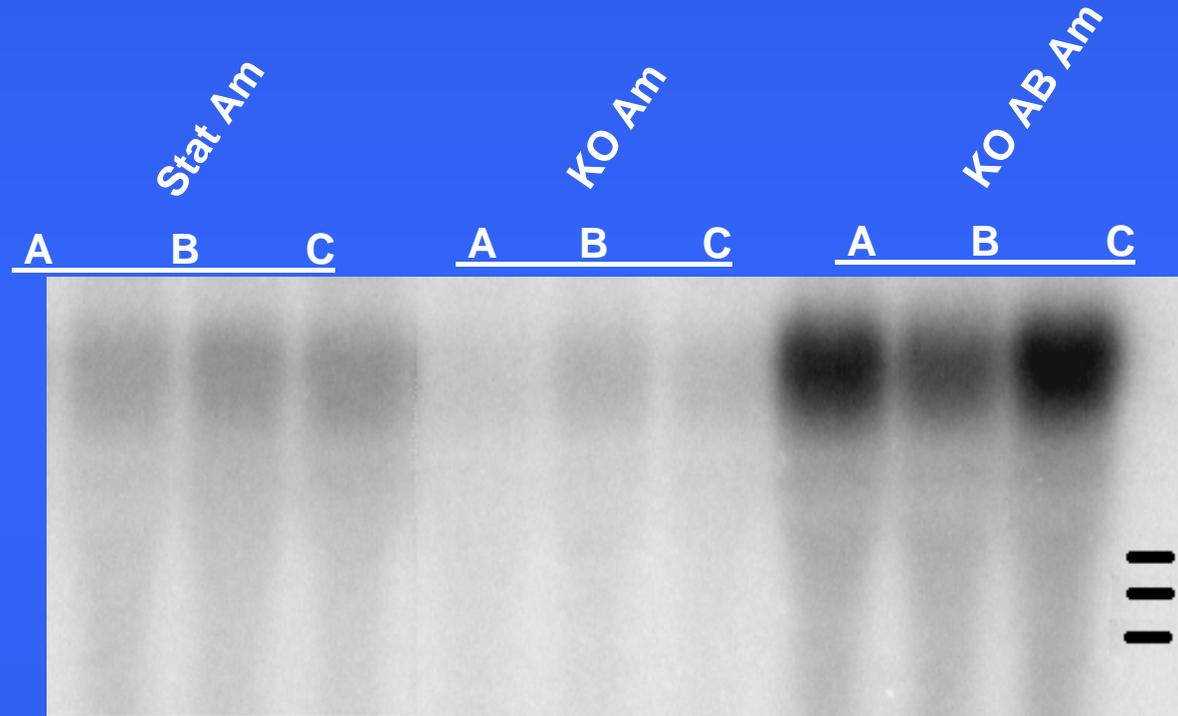
Infection of cultured human macrophages



J. Biol. Chem. (2004) 279: 25703

1. Loss of infectivity *in vitro* by gene deletion
2. Potential vaccine candidate for *in vivo* testing

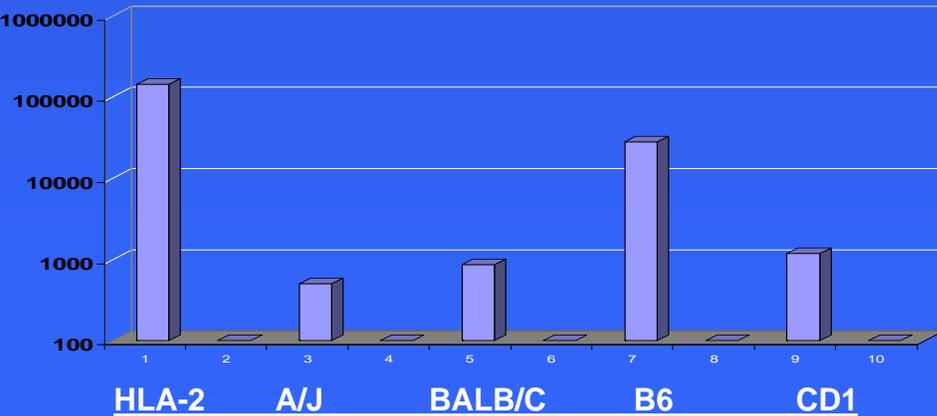
Biomarkers to Monitor Safety of Attenuated Leishmania Vaccine



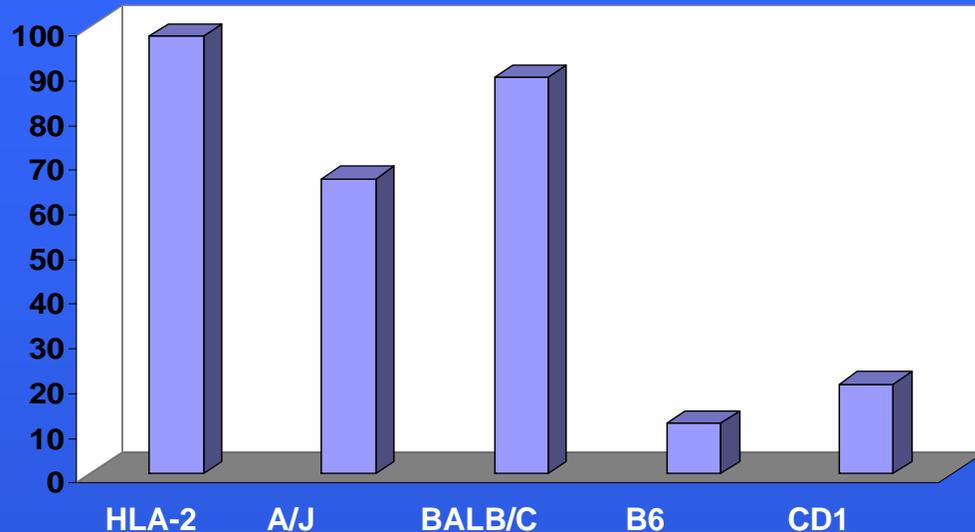
1. Expression pattern comparison of knock-out vs. wild type parasite
2. Identified altered expression of some genes
3. **Expression patterns could be used as biomarkers for vaccine safety**

Efficacy of *Malaria* Peptide Vaccine

ELISA IgG against MAP

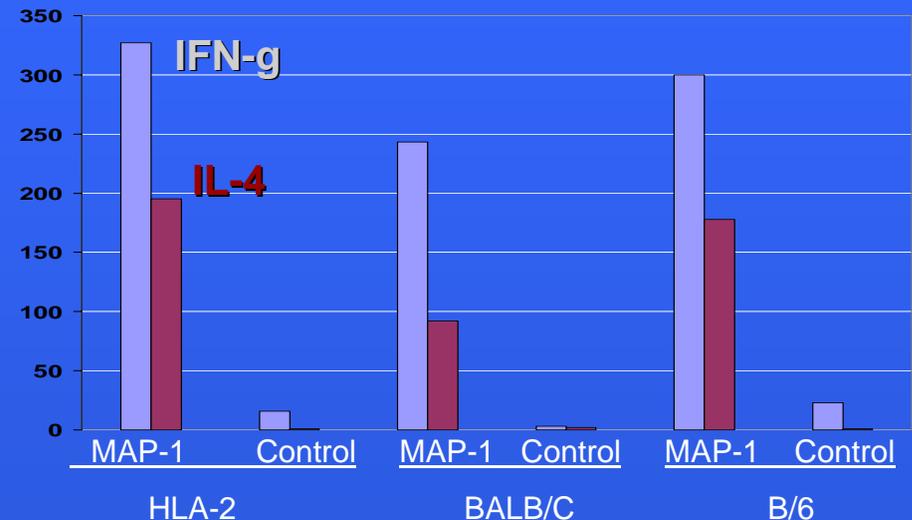


Sporozoite inhibition assay

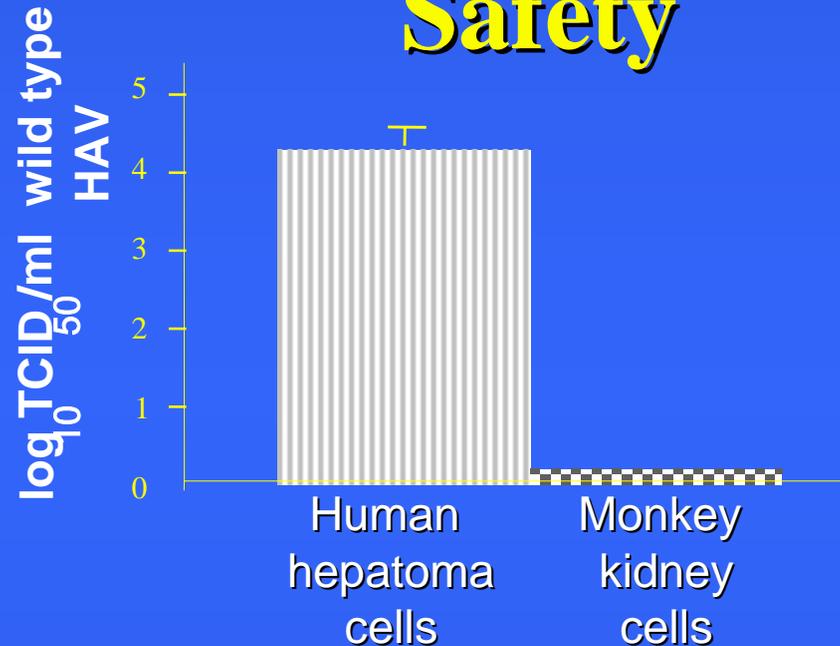


- ◆ Branched-chain MAP representing CD4, CD8 and B cell epitopes from sporozoite, liver form and erythrocytic
- ◆ Humoral response: moderate to high
- ◆ Cellular response: moderate to high
- ◆ Growth inhibitory effect against liver stage parasites: variable

ELISPOT: IFN-g and IL-4 secreting T cells

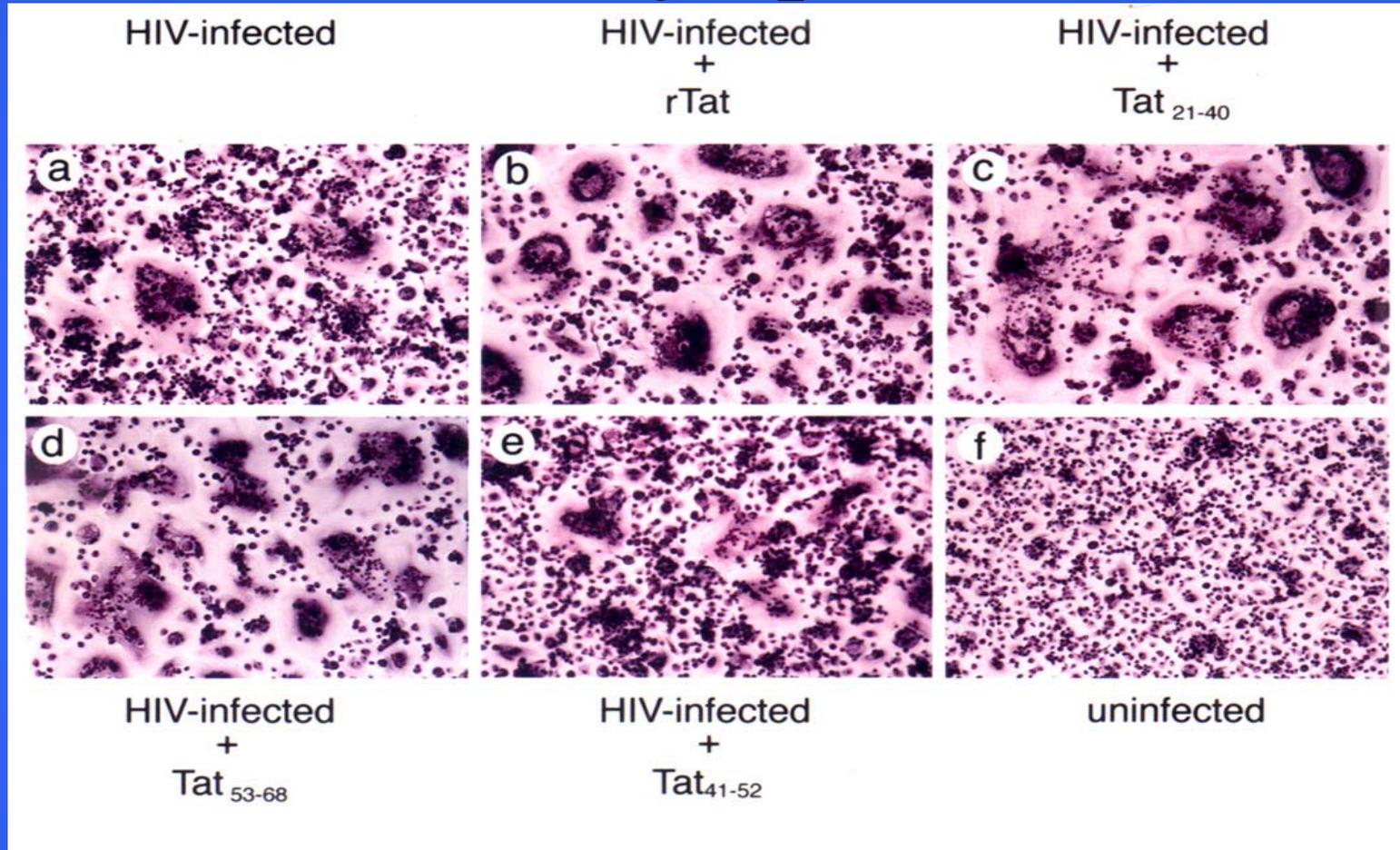


Growth of Wild-Type Hepatitis A Virus in Cell Lines as Aid in Diagnosis and Vaccine Safety



- Production of a new generation of cost-effective attenuated vaccines for the control and eradication of HAV
- As an aid in the diagnosis of HAV infection in blood and blood products

HIV Therapeutics: Tat-Induced Cytopathic Effects



Journal of Immunology 163: 15-20 (1999)

1. Identification of Tat protein domains which enhance HIV replication
2. Immunization with such peptides could be used as therapeutic vaccine

C. Manufacturing Issues (TSE): **US Public Health Impact**

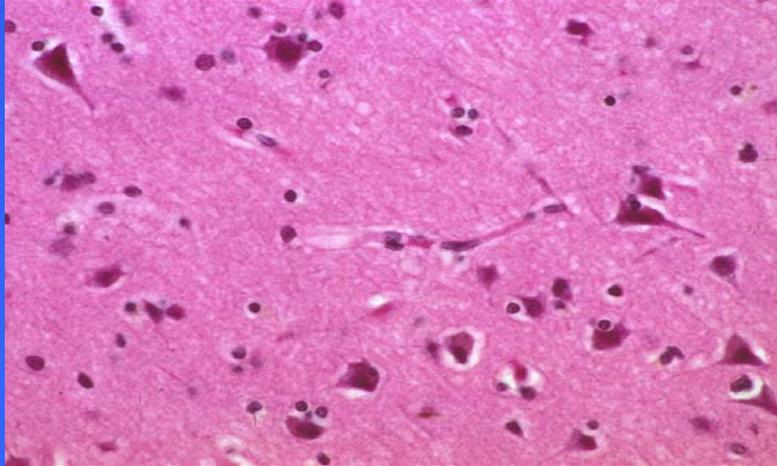
- **Millions of Americans visited or lived in UK, other countries with substantial prevalence of bovine spongiform encephalopathy (BSE), uncertain food chain protection**
- **Number of persons infected with vCJD agent uncertain**
- **Uncertainty regarding silent incubation period of vCJD**
- **TSE contaminated blood products caused fatal infections**
- **Documented vCJD T-T cases**

C. Manufacturing Issues (TSE): Regulatory and Scientific Challenges:

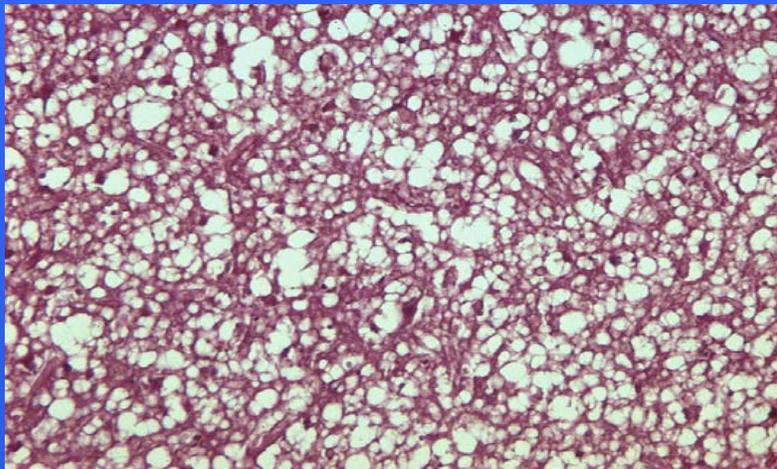
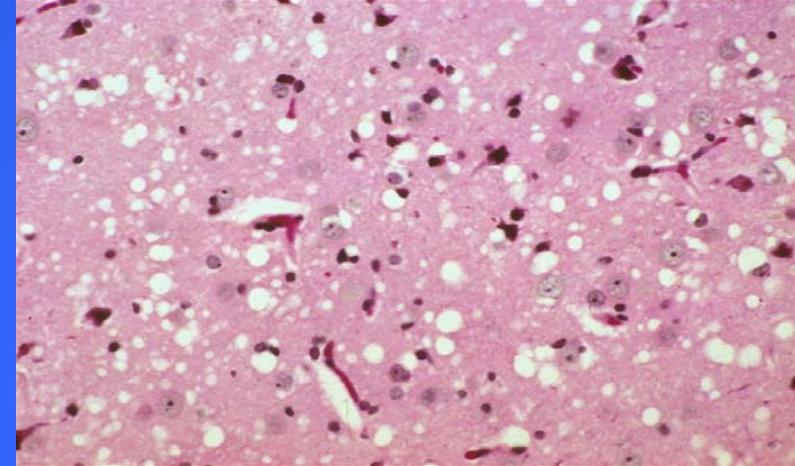
- ◆ **Establish criteria for accepting TSE infectivity removal claims for processes used in blood collection, component processing, and for manufacture of blood derivatives, vaccines and other biologic products**
- ◆ **Establish criteria for accepting TSE infectivity decontamination claims**
- ◆ **Gaps in knowledge for critical path development of TSE detection assays**

Transmissible Spongiform Encephalopathies (TSEs or Prion Diseases)

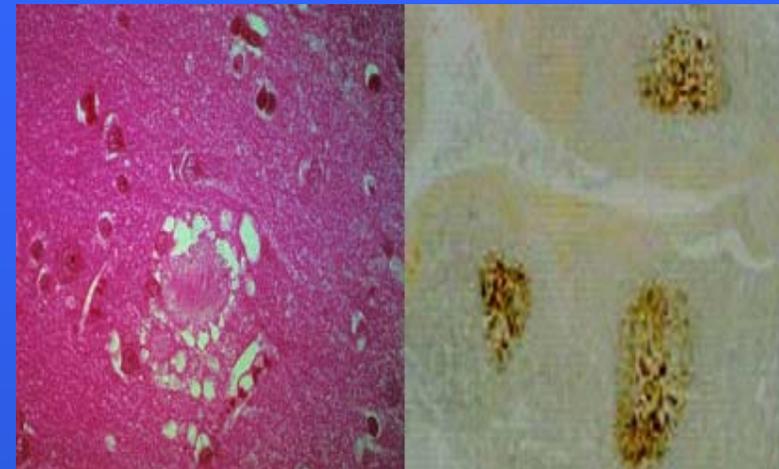
Control



Early stage of CJD

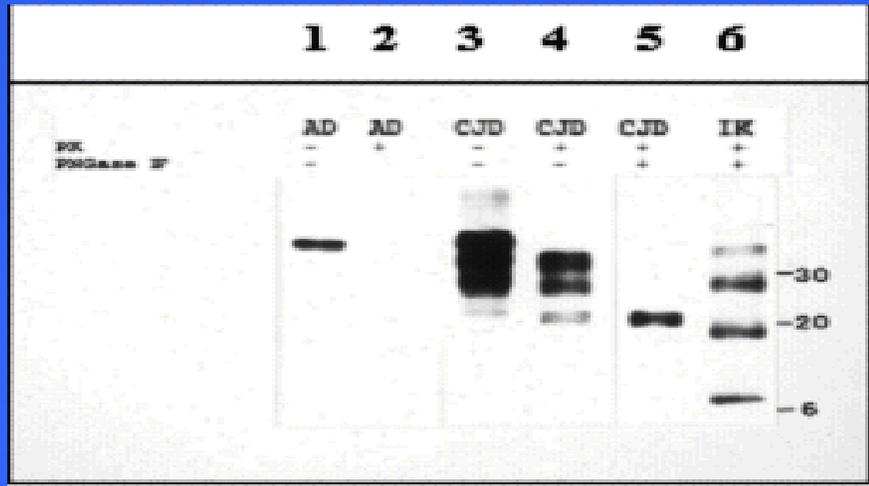


Late stage of CJD

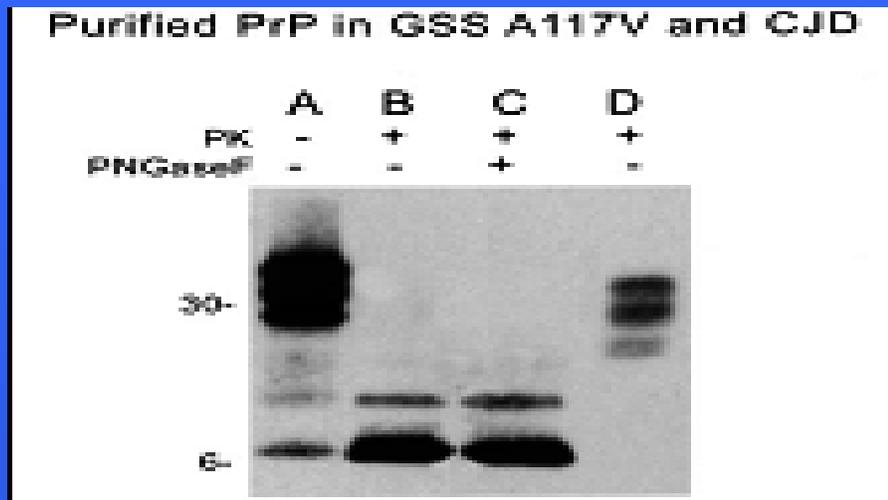


vCJD

Assuring Human-Derived/ Animal-Derived Materials Free of TSE Agents

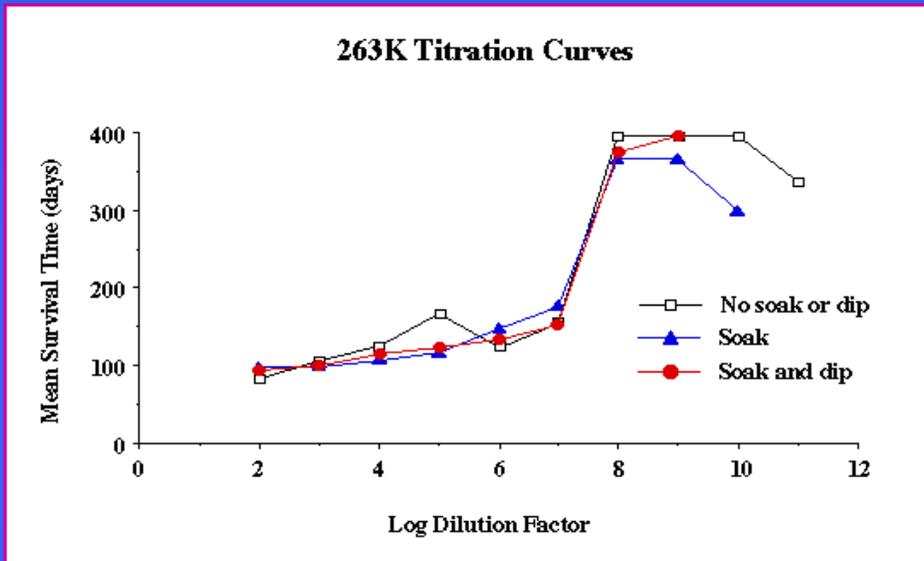


❖ Testing tissues for PrP infectivity



➤ PrP infectivity only associated with HMW protease-resistant forms

Assuring Manufacturing Facilities and Equipment Free of TSE Agents



Chem + autocl + hot alk detergent ultrasonic cleaner	Animals Scrapie+/Total	Objects Scrapie+/Total
Ia 1N NaOH@121Cx30'	0/40	0/10
IIa 6% NaOCl→121Cx30'	0/40	0/10
Ib 1N NaOH@134Cx90'	1/37 (245 da)	1/10
IIb 6% NaOCl→134Cx90'	0/40	0/10

□ Validation of WHO-recommended TSE decontamination protocols

✓ NaOH or NaOCl plus autoclaving removed scrapie infectivity from glass and steel surfaces

Future Directions

- ◆ Evaluation of new technologies to allow rapid, sensitive and multiplex detection of new and emerging blood-borne pathogens (HIV, Hepatitis, WNV, Parasitic and TSE agents)
- ◆ Prevalence and diagnostic significance of HIV variants and HBV mutants
- ◆ WNV and other emerging agents: infectivity in blood, standards development for validation of assays
- ◆ CJD/vCJD detection and decontamination

Future Directions (Cont...)

- ◆ **Biomarkers to predict vaccine safety and immunogenicity of attenuated parasite vaccines**
- ◆ **Cell culture for human hepatitis viruses as an aid in diagnosis and to evaluate vaccine safety**
- ◆ **Assessment of efficacy of viral (HIV, WNV) therapeutic vaccines**

**Mission Relevant Research in viral,
parasitic and TSEs diseases
improves OBRR/CBER's ability to
evaluate the safety and efficacy of
blood and blood products and
vaccines**

Acknowledgments

- ◆ Safety and efficacy of Human Immunodeficiency virus (HIV), Human T-cell leukemia virus (HTLV), West Nile virus (WNV) and smallpox vaccine donor screening and diagnostic assays (PIs: **Indira Hewlett, Subhash Dhawan, Andrew Dayton, Maria Rios**)
- ◆ Safety and efficacy of donor screening assays for hepatitis and emerging viruses (PIs: **Gerardo Kaplan, Deborah Taylor, Edward Tabor**)
- ◆ Safety of blood from the risk of transmission of parasitic agents and safety of such vaccines (PIs: **Hira Nakhasi, Sanjai Kumar, Alain Debrabant**)
- ◆ Safety of biologics from Transmissible Spongiform Encephalopathies (TSE) (PIs: **David Asher and Pedro Piccardo**)