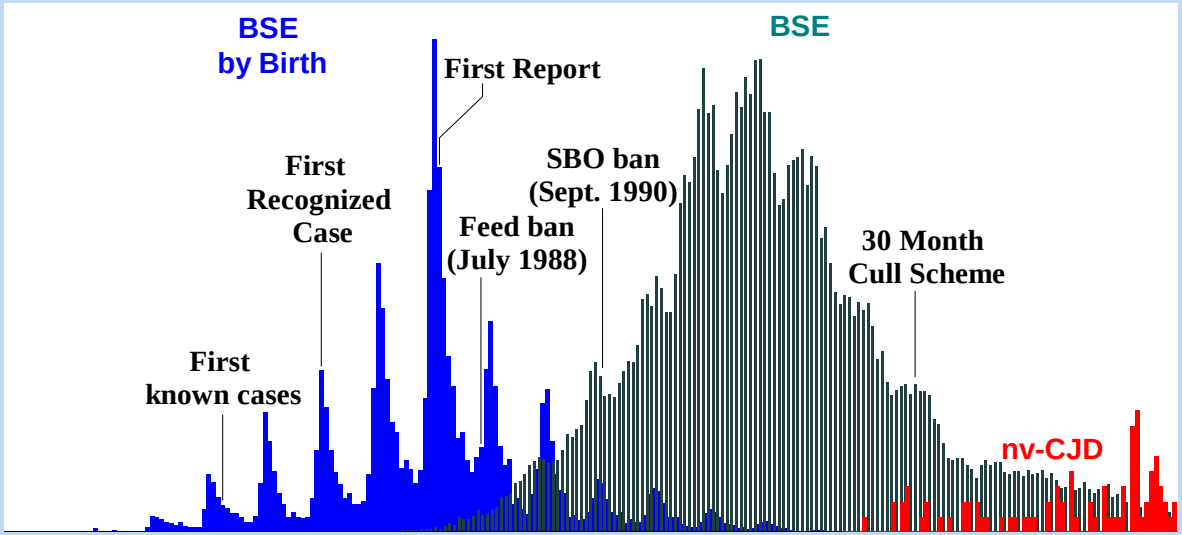
PHS Advisory Committee on Blood Safety & Availability

Bethesda, Maryland
May 17, 2005



Removal of TSE Infectivity from Blood and Blood Products by Adsorption

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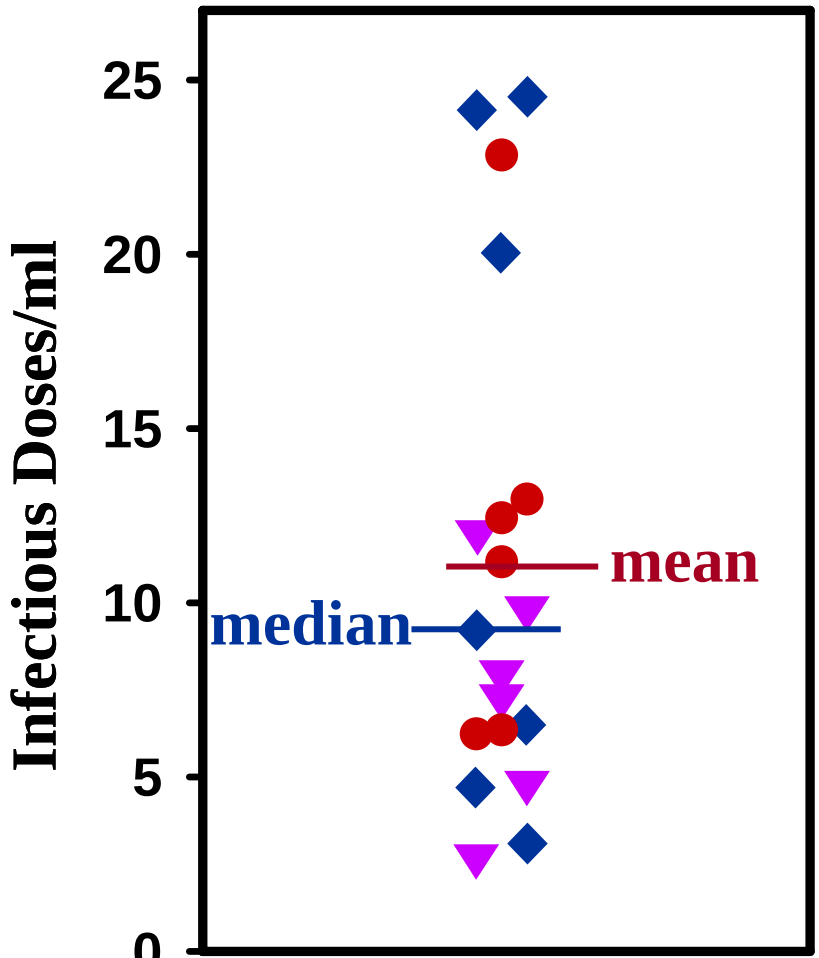
PRDT

Pathogen Removal and Diagnostics Technologies, Inc.

- **Founders**
  - **Dave Hammond, Ph.D.**
  - **Ruben Carbonnel, Ph.D.**
  - **Robert Rohwer, Ph.D**
- **Joint Venture**
  - **American Red Cross**
  - **Prometic Corp.**
- **Manufacturing and Marketing Partner**
  - **MacoPharma**

Titer of TSE Infected Blood
in Clinically Affected Animals



Pools

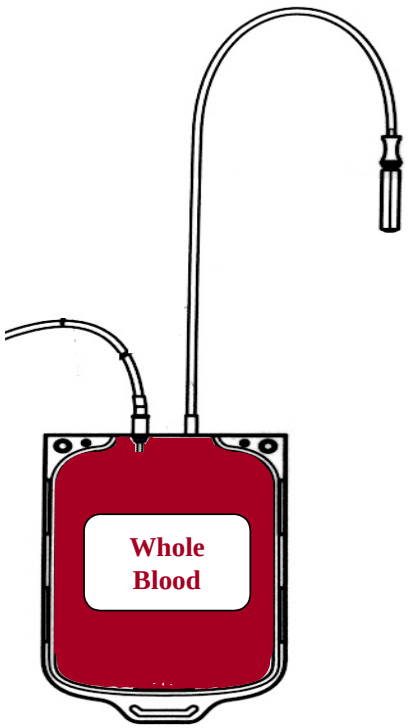
Individual

Splenectomies

Blood vs Brain

Tissue	Infectivity Titer
Blood	10 ID/ml
Brain	10,000,000,000 ID/ml

Infectivity in a Unit of Blood

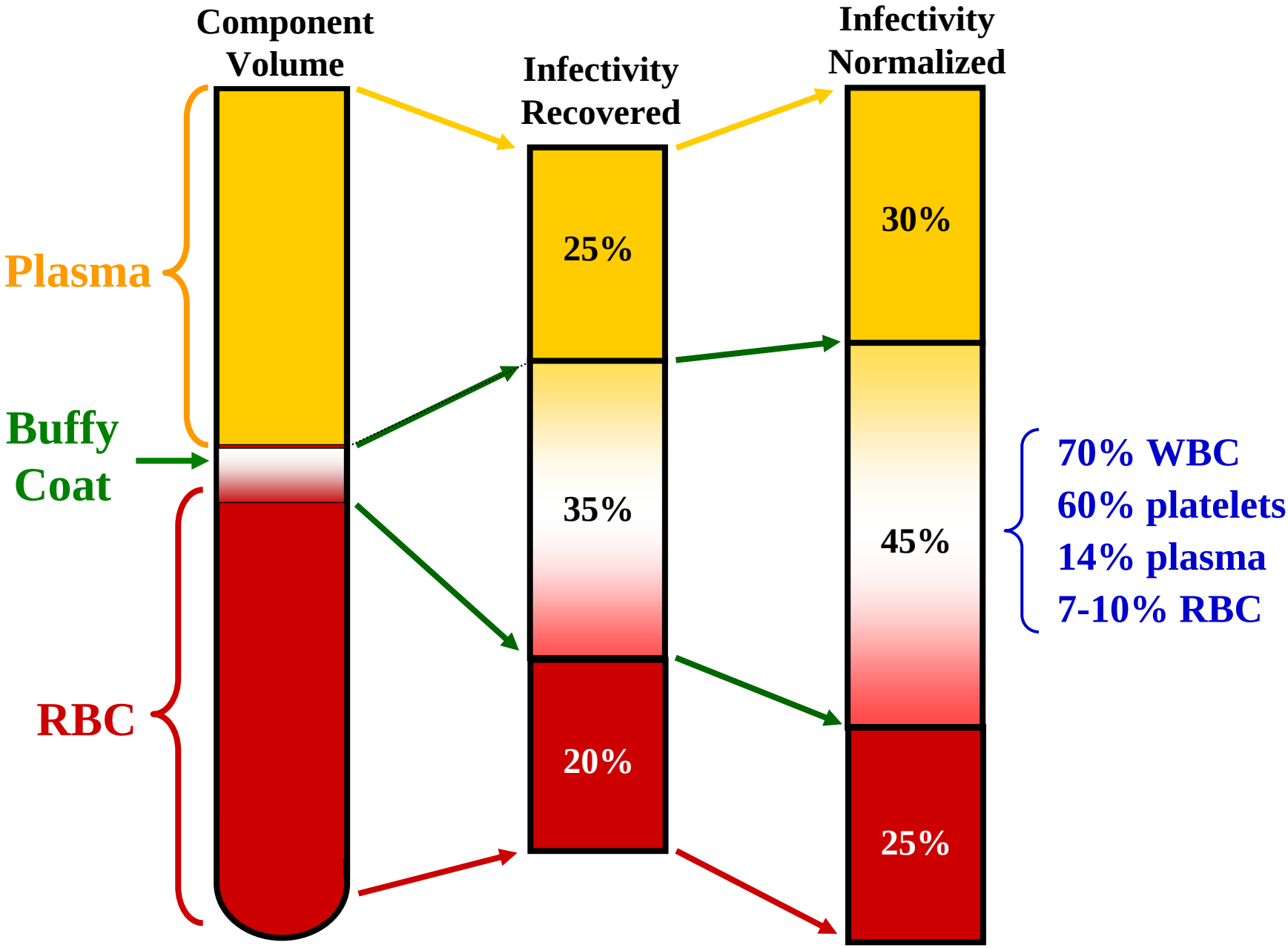


Average

- 10 ID/ml
- 450 ml/Unit
- 4500 ID/Unit

3 to 4 $\log_{10}$ ID/unit

Distribution of Infectivity in Blood Components



Control of TSE Pathogens

- **Sourcing/Deferrals – moving target**
- **Screening – technically problematical for blood**
- **Inactivation – incompatible with product**

or

Risk Substitution instead of Risk Reduction

- **Removal – low risk, technically accessible**

Advantages of Adsorption for TSE Risk Reduction

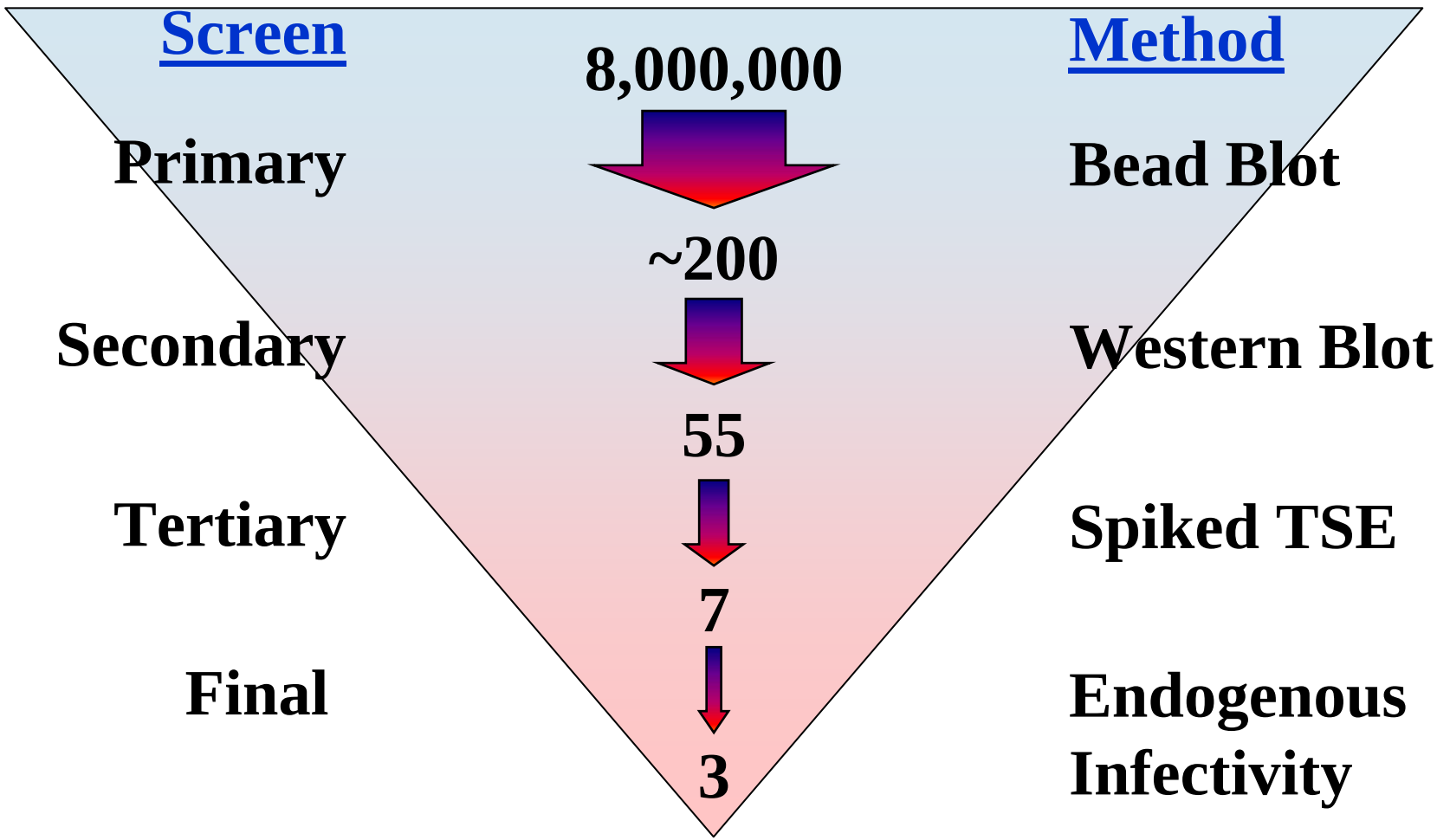
- Removes infectivity that can not be detected with diagnostics
  - Clinical disease for blood
  - Preclinical disease for brain
- Discrimination of abnormal from normal PrP is not necessary or even desirable
- More comprehensive than a diagnostic
- May be less costly than diagnostics

Critical Elements of Combinatorial Chemistry

- **Size of structure libraries**
  - **No theoretical limit**
- **Diversity of structures in library**
- **Screening method**
  - **Relevant**
  - **Stringent**
  - **Most serious limitation**

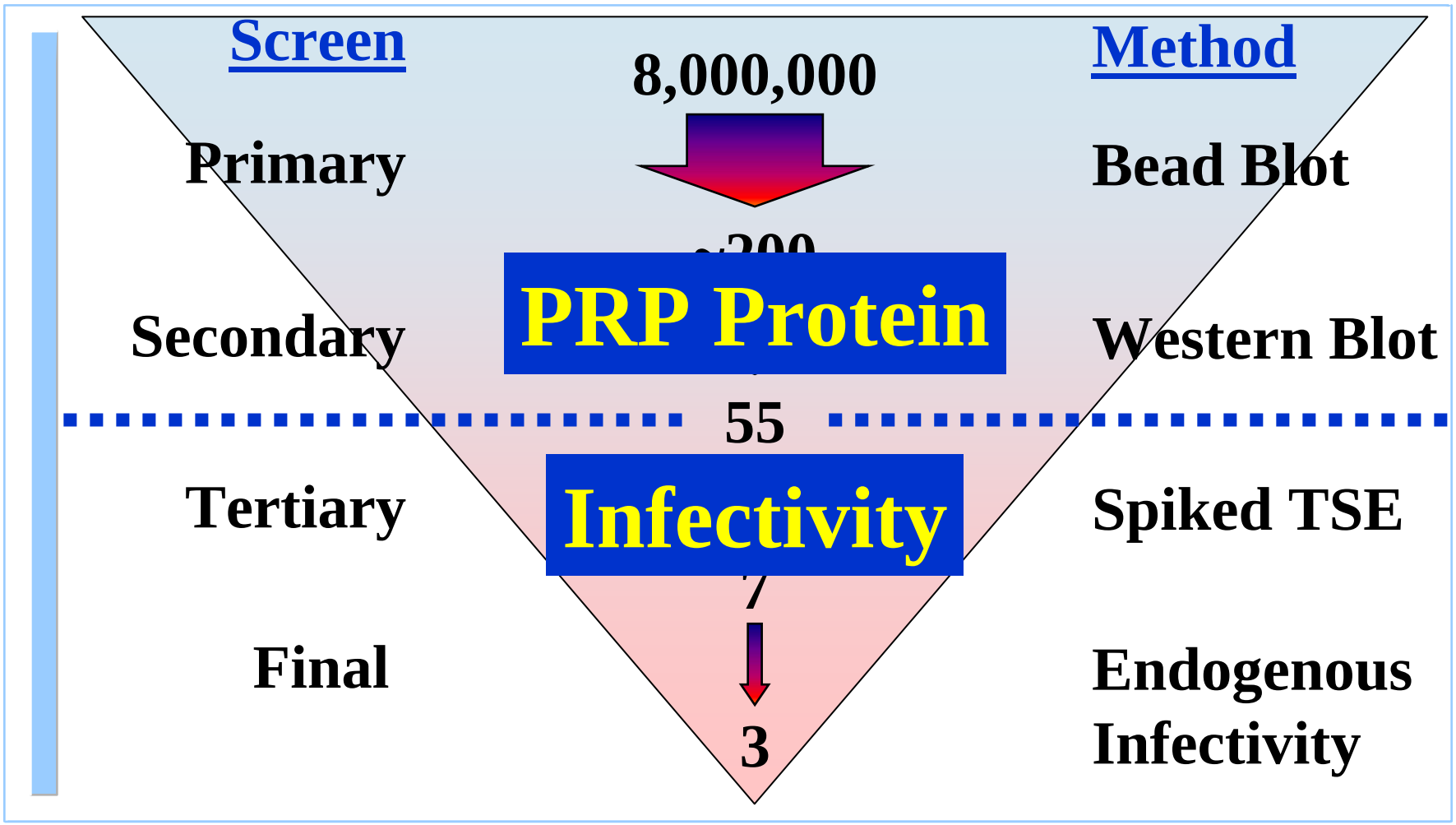
Screening

Library: 64,000,000 combinations

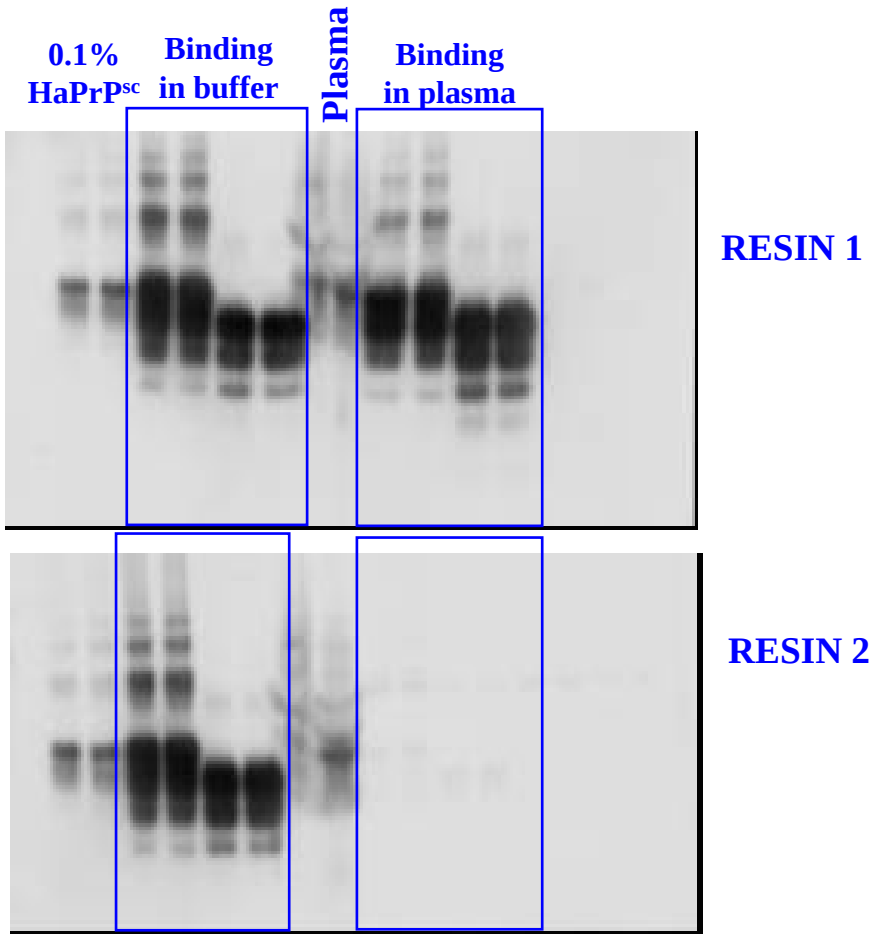


Screening

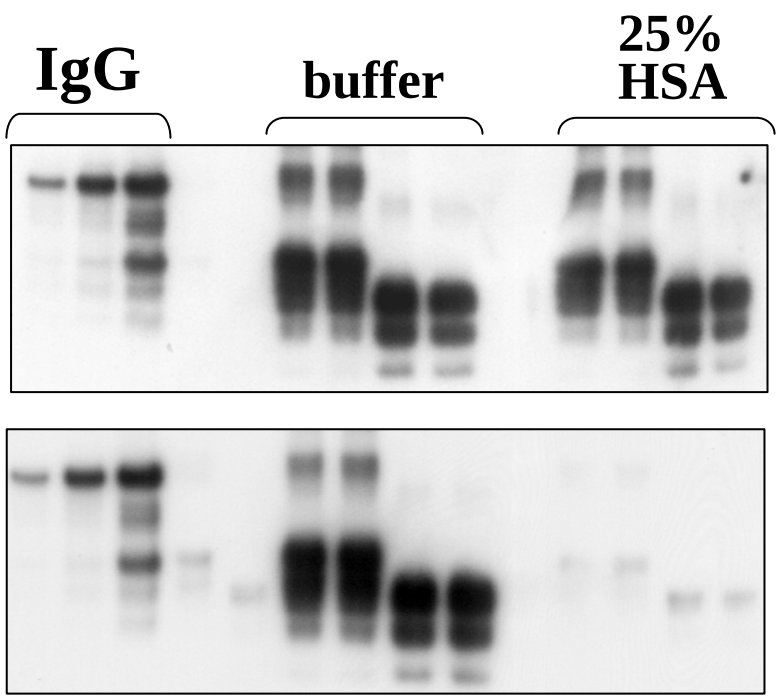
Library: 64,000,000 combinations



Binding in Plasma



Specificity of binding



Screening - Ideal

- vCJD infected blood from a human patient
  - Full scale
  - Limiting dilution titration in a highly susceptible rodent.
- Strengths
  - Actual implementation
  - Human PrP & infectivity
- Weakness
  - Sufficiently sensitive mouse does not exist
  - Full units of vCJD blood have not been available
  - Variability of human blood

Infectivity Experiments

- **Removal of hamster brain infectivity spiked into RBCs**
  - **Primary screen**
  - **Incubation time measurements**
- **Removal of endogenous hamster infectivity from whole blood**
  - **Validation of resin and proof of concept**
  - **Limiting dilution infectivity assays**

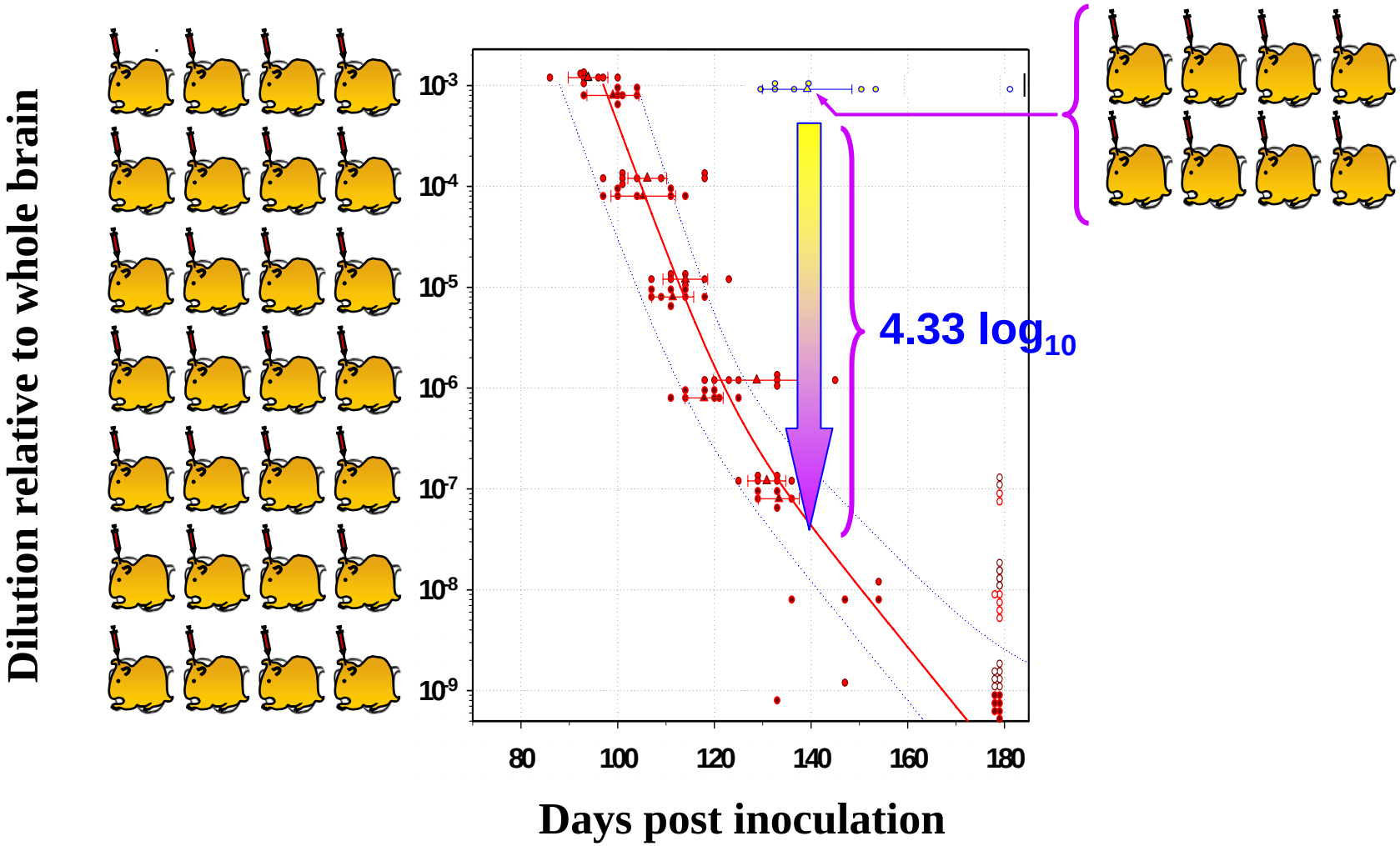
Spiking Experiment

- Human leukoreduced RBCC
- Hamster scrapie brain derived spike – highly dispersed
  - Is not removed by unsubstituted resin
- Large uniform pool distributed to multiple devices
- Challenge each device with a full unit
- Incubation time measurement
- Looking for dramatic reductions

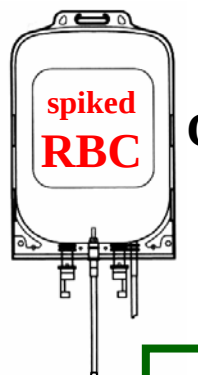
Incubation Time Bioassay

Dose Response Standard Curve

Test Group



RBC spiked with Brain Homogenate

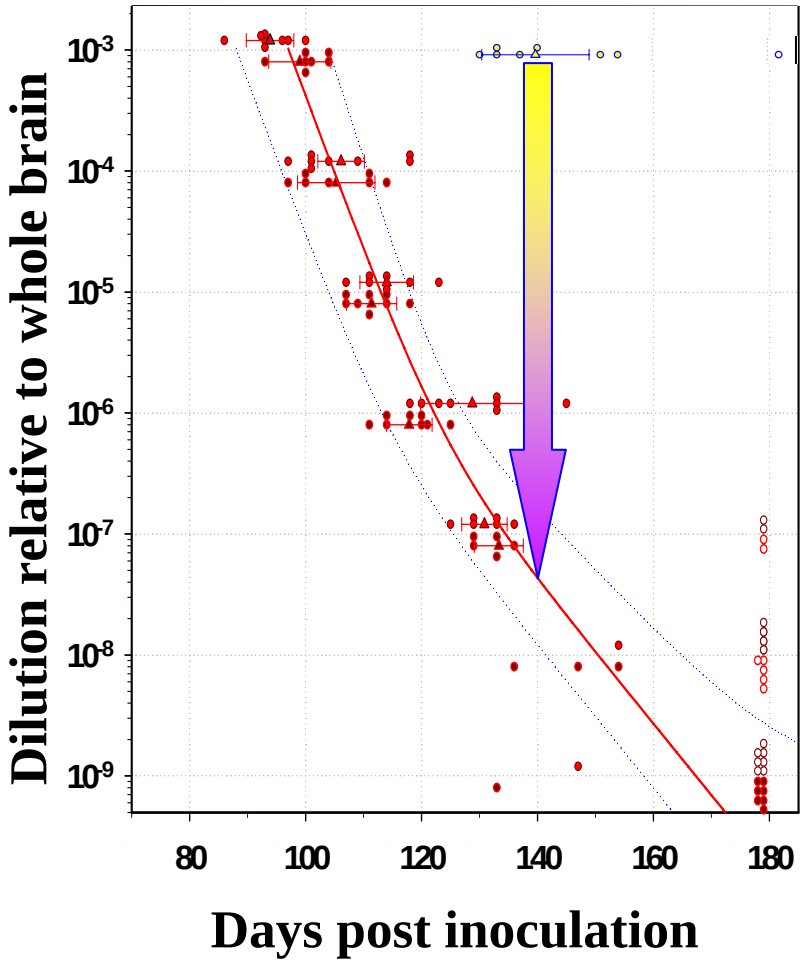


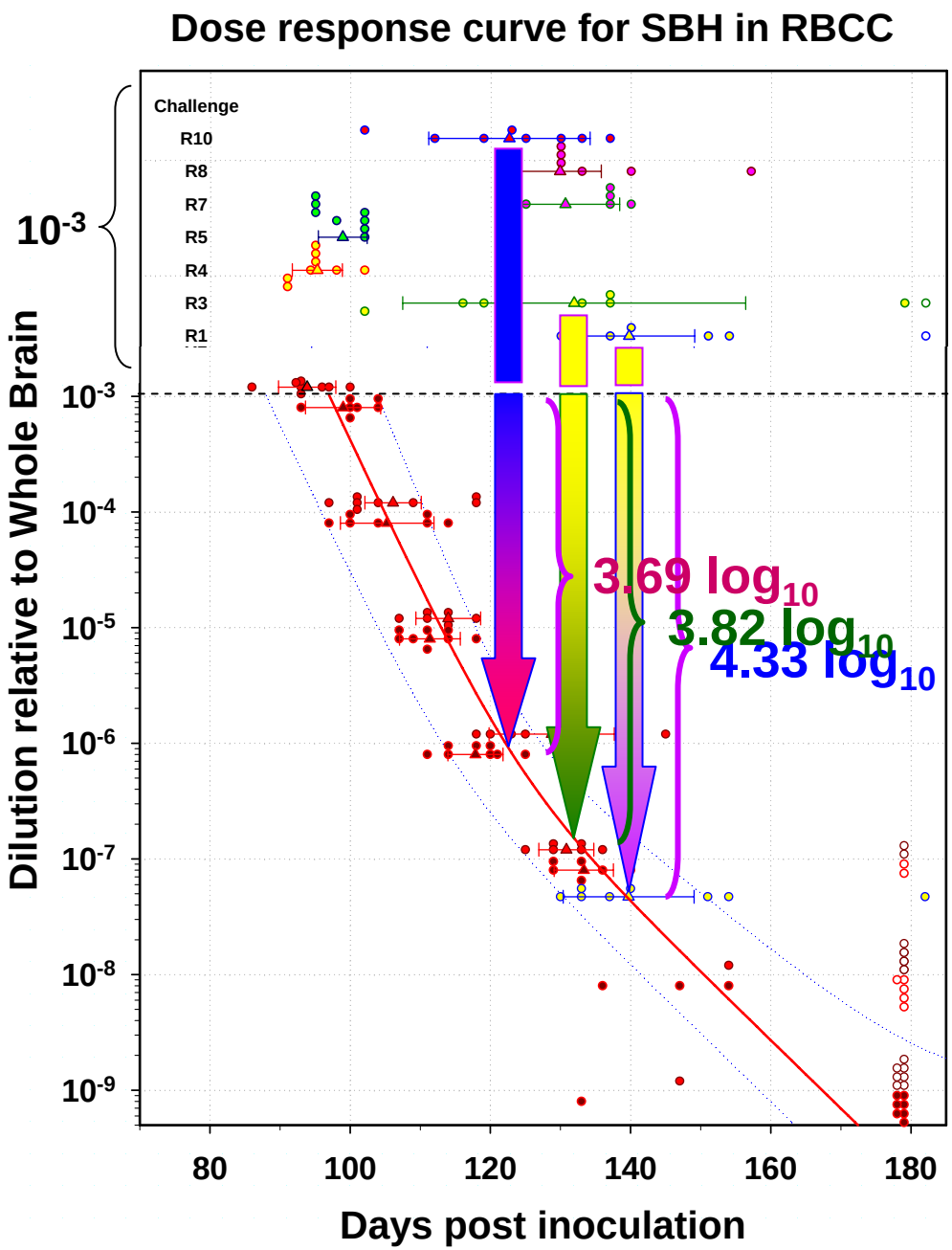
Challenge

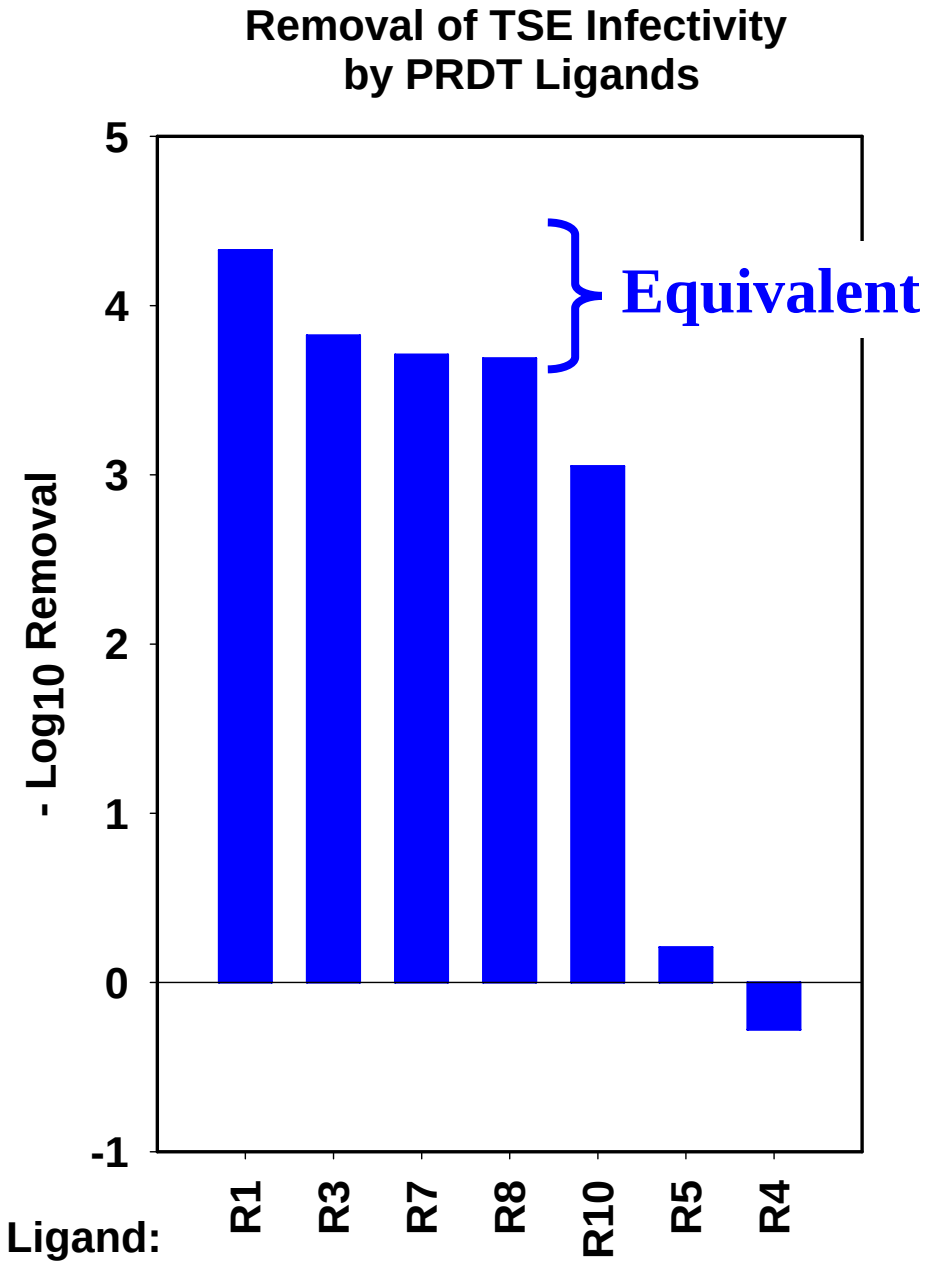
Western blot

Bioassay

Flow Through

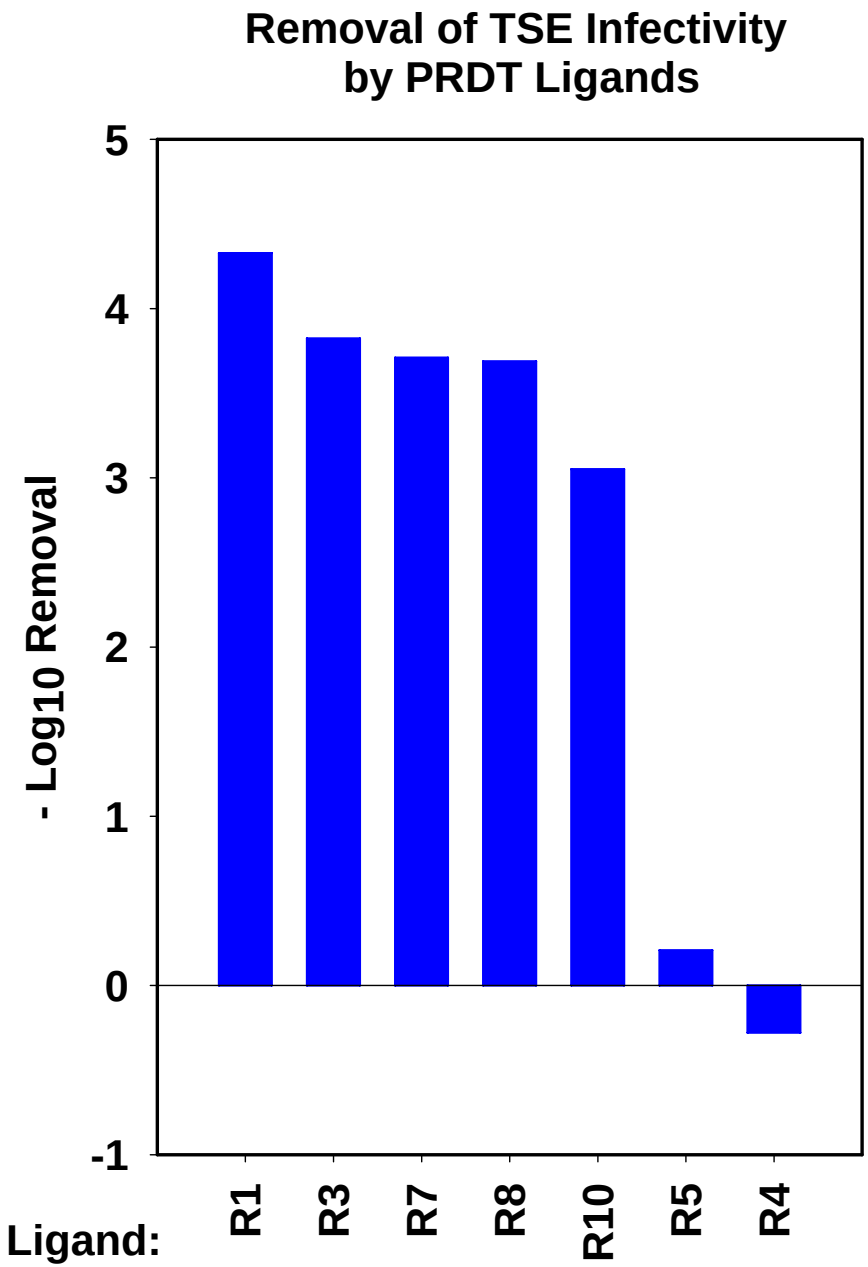



Dose response curve for SBH in RBCC
Challenge
R10
R8
R7
R5
R4
R3
R1
10-3
Dilution relative to Whole Brain
10-3
10-4
10-5
10-6
10-7
10-8
10-9
3.69 log10
3.82 log10
4.33 log10
80
100
120
140
160
180
Days post inoculation



Overloaded Challenge

- **Blood titer: 10 ID/ml**
- **Challenge titer: 10^6 ID/ml**
- **Limit of detection: 20 ID/ml**
- **Maximum Removal: $5x10^4$ ID/ml**



Overloaded Challenge

- 1 part per 50,000 escapes removal
- If blood infectivity is in the same proportion as brain infectivity there is a wide margin of safety.
- If blood is enriched in the minor unremoved fraction it will reduce the effectiveness proportionally.
- It is essential to establish the removability of endogenous blood infectivity.
  - As a proof of principle
  - As a validation of relevance

Endogenous Infectivity Removal

- Samples
  - Whole Blood
  - Leucoreduced blood
  - Five Resin Samples
  - One control
- 800 animals
- 280 days / 550 days

PRDT Device Format

- The lead ligand is chemically immobilised to an inert resin support matrix
- The resin is in turn sandwiched between two membranes
- Membranes are integrated and placed in an outer housing
- Blood is passed over the filter and prion protein removed

Partnership

- **Partnership established with MacoPharma**

  - **One of Europe's leading suppliers of blood collection systems**
  - **Will manufacture and supply the prion reduction filter**
  - **Is involved in the end stage development**

Status – Filter Characteristics

- **Lead ligand and resin defined – extremely high affinity for prion protein (> 10^9 Ka)**
- **Membrane defined**
- **Binding and removal of prion protein from; rodent brain, sp CJD and vCJD human brain demonstrated**
- **Utility of product demonstrated for; red blood cell concentrate, whole blood and plasma (*in vitro*)**
- **Demonstrated 99.99% removal of brain derived infectivity**
- **No impact of product on RBC, plasma proteins or platelet activation**

Status – Filter Characteristics

- Scale up of manufacture – ongoing
- CE marking anticipated 2005
- UK and Irish Blood Services are currently evaluating the technology

Removal = Concentration

- **A key element in diagnostic development**

Test Materials → **Concentration and Capture** → **Discrimination of PrP^{res}** → **Detection** → **Validation**

- **A concurrent objective**

PRDT Concentrator for Diagnostics

- **Will increase the sensitivity of any assay**
- **Essential for blood-based assay**
- **Generic front end for existing assay formats**
- **Novel assays based on the device itself**

The End