

# A Three R's Approach to Development of an *in vitro* Potency Assay for a Bovine *Leptospira* Vaccine.

## Jeffrey Galvin

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# Introduction

- Background information on Spirovac<sup>®</sup> vaccine
- Strategies for “Refinement” and “Reduction” of Animals
- “Replacement”: Successful Approval of an *in vitro* ELISA
  - Validation Data
  - Link(s) to Host Animal Efficacy (Qualification)
  - Pros/Cons for the Spirovac<sup>®</sup> *in vitro* ELISA
- Development Timeline

A second generation leptospirosis vaccine containing inactivated *Leptospira borgpetersenii* serovar hardjo type hardjo-bovis adjuvanted with aluminum hydroxide. The vaccine has label indications for use in healthy cattle 4 weeks of age or older, including pregnant and/or lactating cows and heifers, for the prevention of infection caused by type hardjo-bovis, and urinary shedding for up to 12 months. Also aids in the prevention of fetal infection.

## Key Attributes

- 2mL dose product for use against strains of type hardjo-bovis
- Safe for use during pregnancy
- 12 month Duration of Immunity (annual booster)
- Prevents Colonization and Shedding



# Hamster Model– Refine....

- CFR-like Model

- Single Immunization
- Lethal challenge
- Challenge material sourced from liver
- ~28 days
- Variable: Death

- Refined Model

- Single Immunization
- Non-Lethal challenge
- *In vitro* challenge (cryopreserved)
- ~56 days
- No clinical signs of Leptospirosis
- 4 weeks of *in vitro* culture
- Variable: Presence of cultivable leptospira

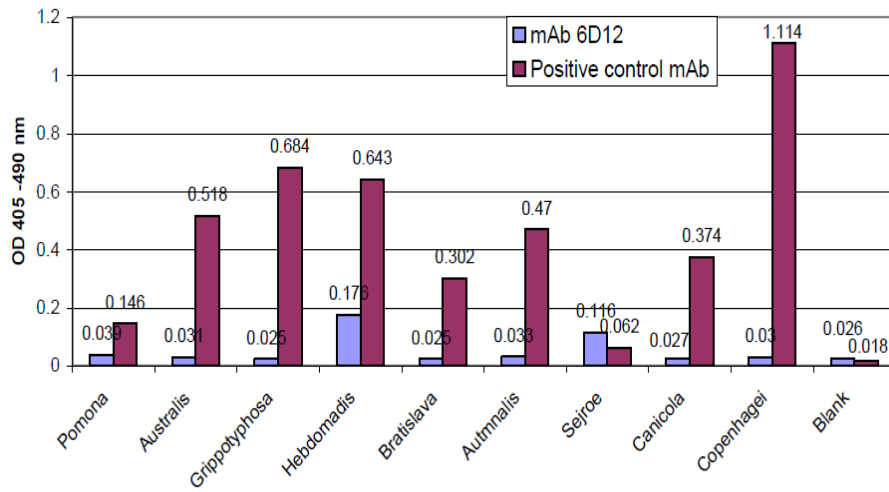


Reduce

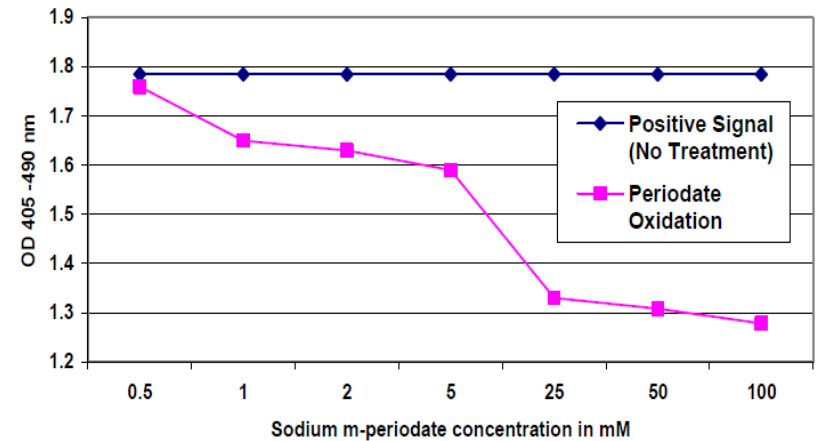
# ELISA Reagents

- Rabbit anti-*L. borgpetersenii* type hardjo-bovis (purified)
- Detection Monoclonal Antibody: 6D12
- Specificity

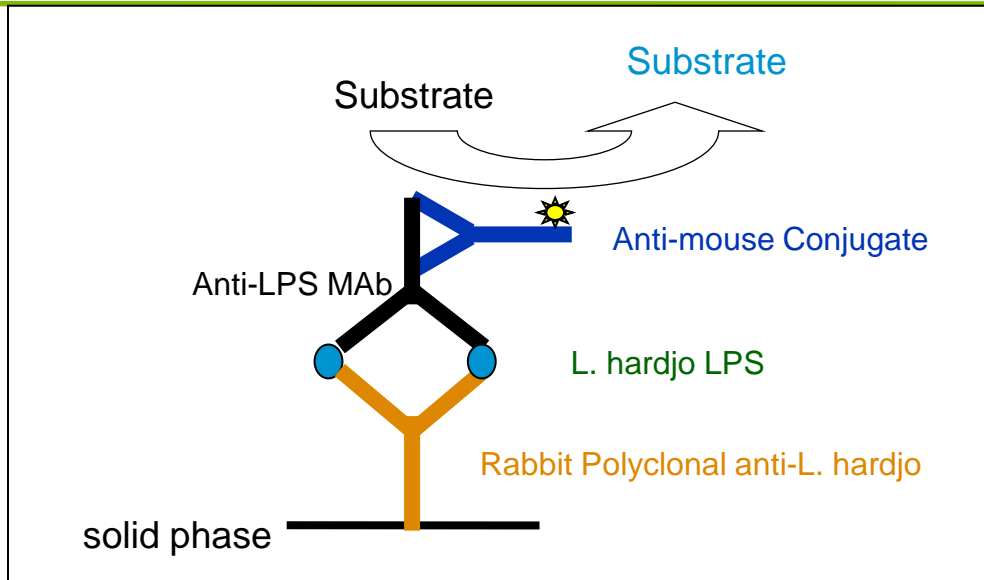
Anti *L. hardjo* mAb 6D12 Specificity in ELISA



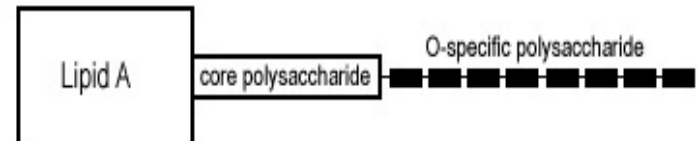
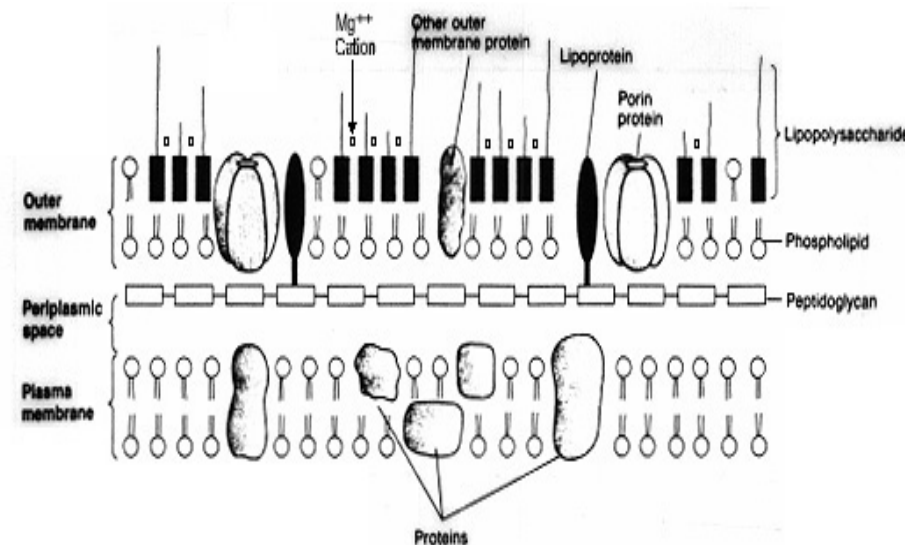
Anti *L. hardjo* mAb 6D12 - LPS Recativity -Sonicate Whole Cell



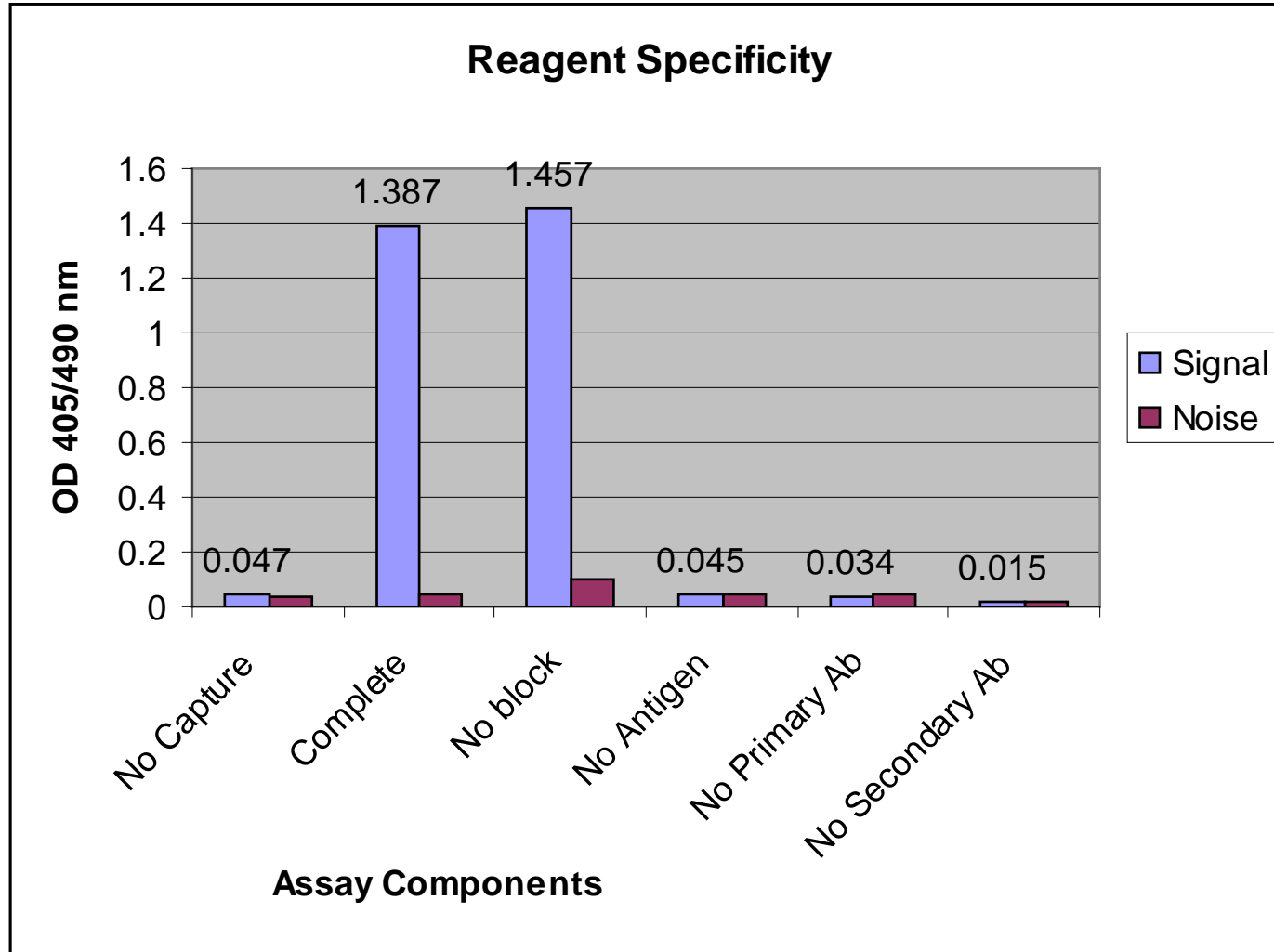
# In Vitro ELISA for *L. borgpetersenii*, serovar hardjo LPS



- ◆ Specific for hardjo
- ◆ Standard antigen is bulk material
- ◆ Suitable assay to measure “antigen” content of bulks/vaccines
- ◆ Standard antigen: lot material (Efficacy study)
- ◆ Positive control: lot material



# In process ELISA (Antigen Fluids) - Specificity



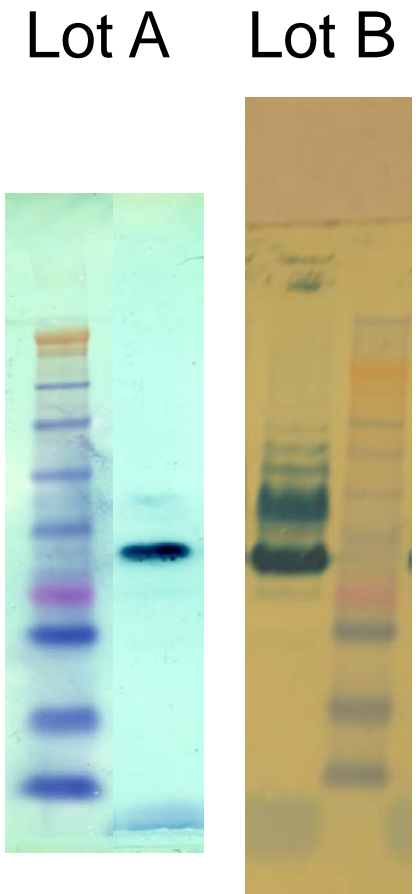
# In process ELISA (Antigen Fluids) - Precision

Technician	Rep	Test date	# 103106	# 0811654	# L420004034	PC #34A	
1 transfer lab	1	Date 1	6165	8052	624	1982	
1 transfer lab	2	Date 1	6208	7883	607	1960	
1 transfer lab	3	Date 2	7272	9773	766	2294	
1 transfer lab	4	Date 2	7526	9918	770	2397	
2 receiving lab	1	Date 3	7078	9875	643	2108	
2 receiving lab	2	Date 3	7234	9963	640	1983	
2 receiving lab	3	Date 4	9315	11494	745	2343	
2 receiving lab	4	Date 4	9310	11715	727	2392	
			<b>Mean</b>	<b>7514</b>	<b>9834</b>	<b>690</b>	<b>2182</b>
			<b>STD</b>	<b>1214</b>	<b>1379</b>	<b>68</b>	<b>194</b>
			<b>%CV</b>	<b>16.16%</b>	<b>14.02%</b>	<b>9.88%</b>	<b>8.88%</b>
					<b>Overall %CV =</b>	<b>12.24%</b>	



# Higher ELISA Values Results in Improved Hamster Potency

Antigen	ELISA RU/dose	Cells/dose	Hamsters
			Positive/Total
Lot A	456	100%	6/10
Lot B	4876	100%	1/10
Lot A	228	50%	7/10
Lot B	2438	50%	3/10
Lot A	91	20%	9/10
Lot B	975	20%	3/10
Lot A	46	10%	9/10
Lot B	488	10%	6/10

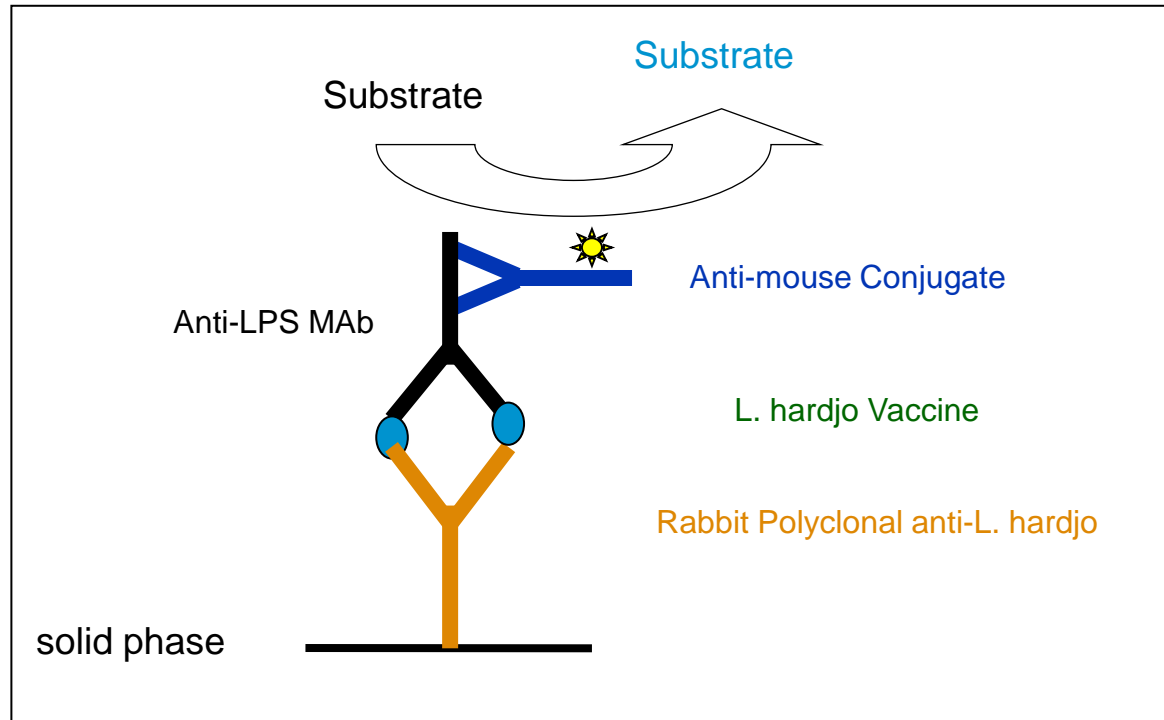


# Reduce....

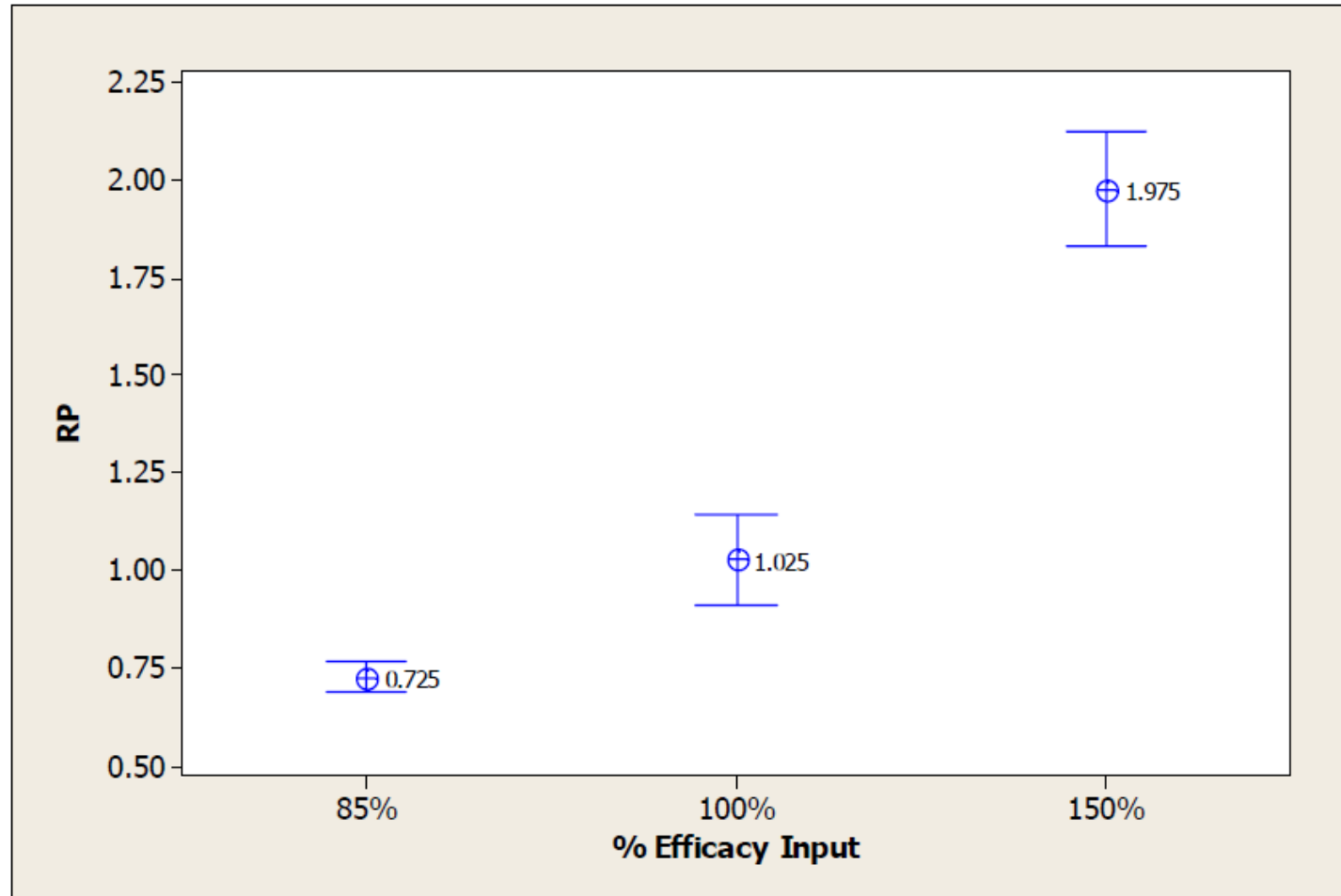
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- ELISA was implemented to measure quality of bulk antigen
- Better correlation to potency
  - Prevent low potency bulk antigens being formulated into vaccines
- Over a three year period:
  - Potency failure rates decreased ~6X
  - ~50% Reduction of numbers of hamsters
    - Avoidance of testing low potency serials
    - Fewer serials for secondary testing

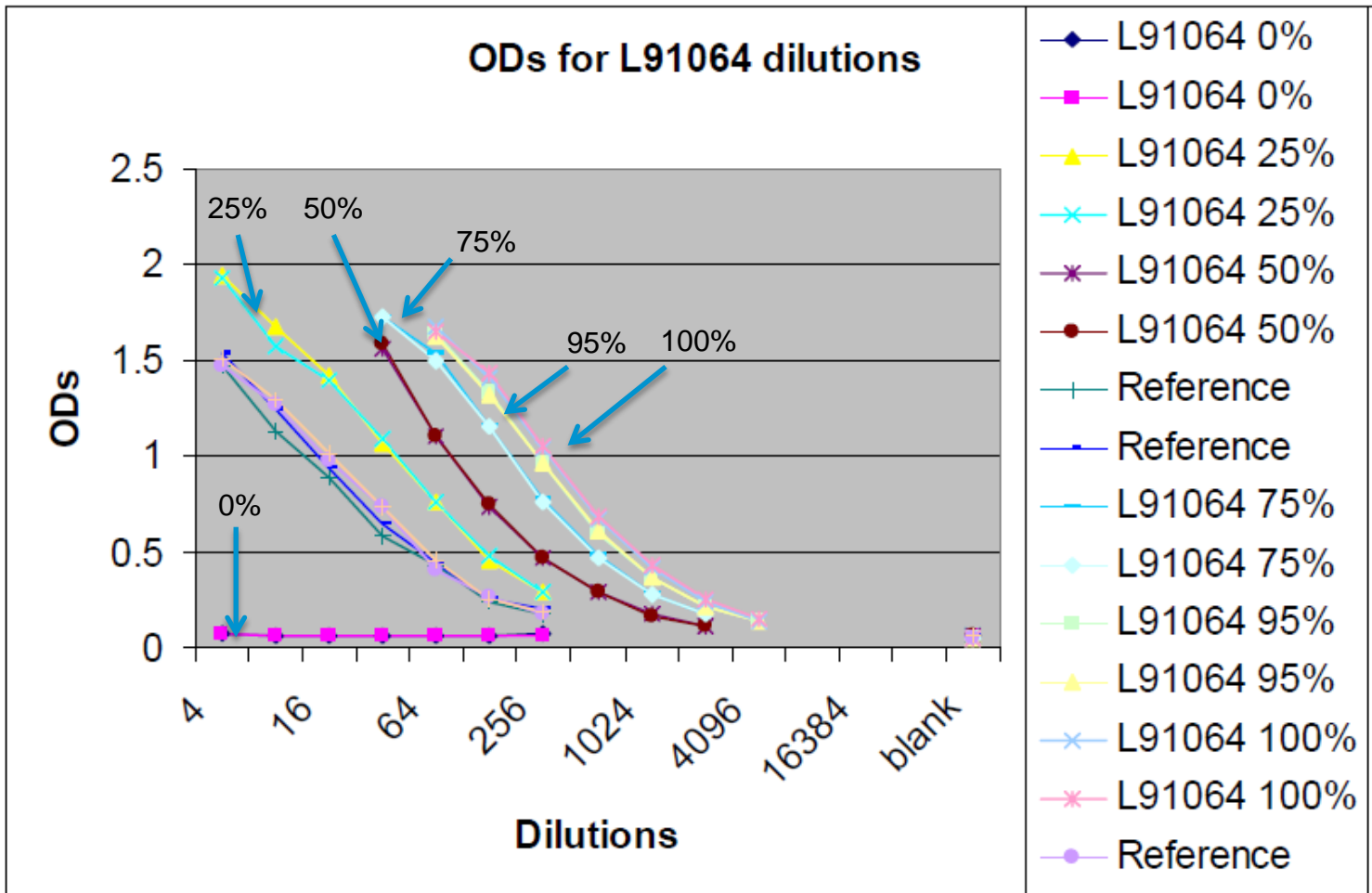
# Final Product Potency ELISA – Replace....



# Validation Data – Dose Discrimination



# Validation Data - Specificity & Linearity



# Validation Data – Precision

	85%	100%	150%
Batch No.	17Nov08A	17Nov08B	17Nov08C
Technician 1	0.7	0.9	1.8
	0.7	1.1	2.0
	0.7	1.1	2.0
<b>Mean</b>	<b>0.7</b>	<b>1.0</b>	<b>1.9</b>
<b>%CV</b>	<b>0%</b>	<b>11%</b>	<b>5%</b>
Technician 2	0.8	1.1	2.1
	0.7	0.9	1.7
	0.6	0.8	1.9
<b>Mean</b>	<b>0.7</b>	<b>0.9</b>	<b>1.9</b>
<b>%CV</b>	<b>14%</b>	<b>15%</b>	<b>11%</b>
Technician 3	0.7	0.9	1.8
	0.7	0.9	1.8
	0.7	0.8	1.7
<b>Mean</b>	<b>0.7</b>	<b>0.9</b>	<b>1.7</b>
<b>%CV</b>	<b>0%</b>	<b>7%</b>	<b>3%</b>
<b>Overall mean</b>	<b>0.7</b>	<b>0.9</b>	<b>1.9</b>
<b>Overall Std Dev</b>	<b>0.1</b>	<b>0.1</b>	<b>0.1</b>
<b>Overall %CV</b>	<b>7%</b>	<b>13%</b>	<b>8%</b>

Values expressed as RP

# Validation Data – Precision (Qualifying Serials)

Serial 1

Technician 1		Technician 2		Technician 3	
Date	RP	Date	RP	Date	RP
15 Jan 2010	10	23 Nov 2009	11	01 Feb 2010	9
	10		10		8
	9				9
01 Feb 2010	8	01 Feb 2010	10	10 Feb 2010	6
	8		10		6
	8		9		7
05 Feb 2010	9	<b>Mean</b>	<b>10</b>	<b>Mean</b>	<b>7.5</b>
	9	<b>%CV</b>	<b>7%</b>	<b>%CV</b>	<b>18%</b>
	10				
<b>Mean</b>	<b>9</b>				
<b>%CV</b>	<b>10%</b>				
<b>Overall mean</b>			<b>8.8</b>		
<b>Overall Std Dev</b>			<b>1.4</b>		
<b>Overall %CV</b>			<b>16%</b>		

Serial 2

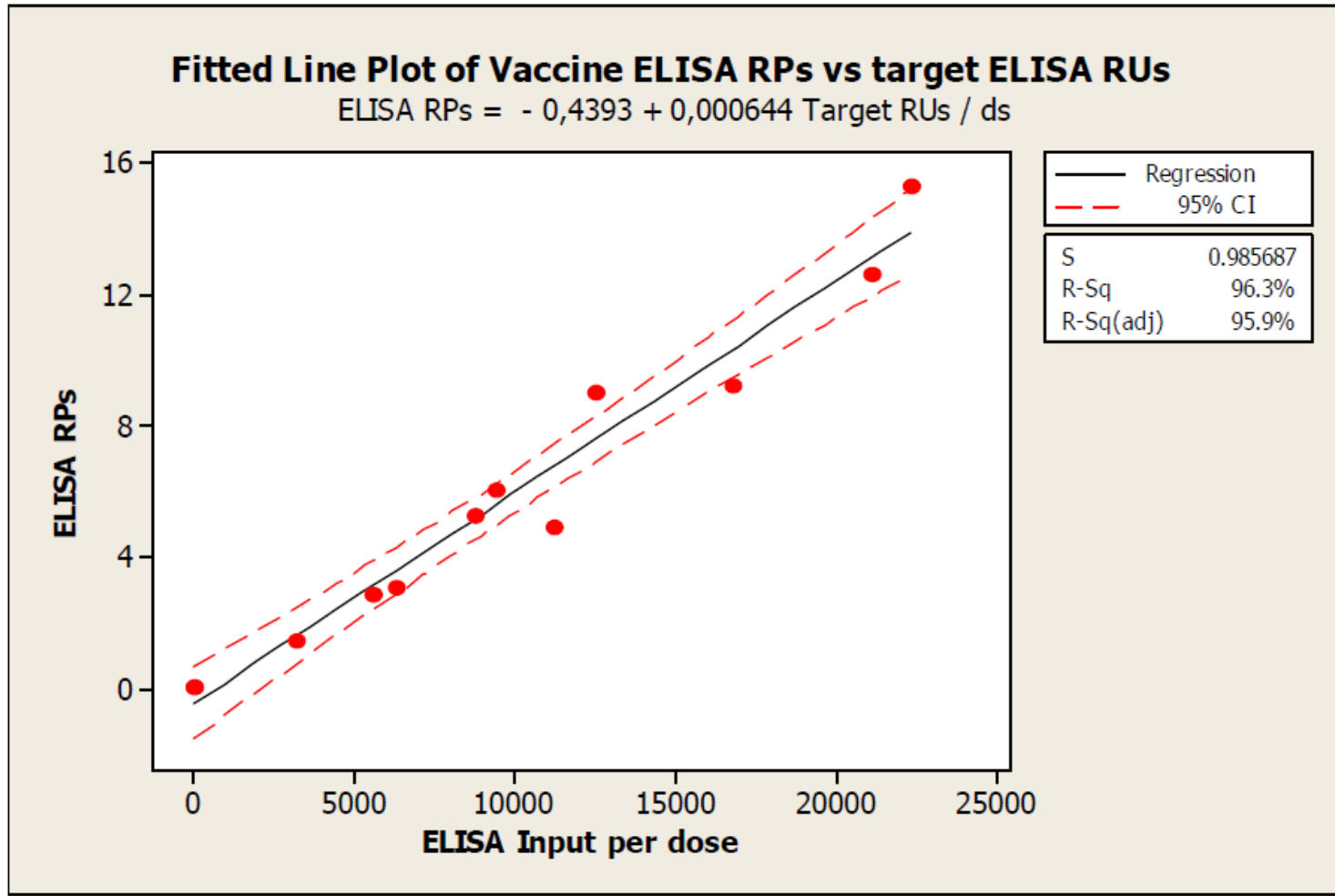
<b>Overall mean</b>	<b>9.1</b>
<b>Overall Std Dev</b>	<b>1.6</b>
<b>Overall %CV</b>	<b>18%</b>

Serial 3

<b>Overall mean</b>	<b>14.1</b>
<b>Overall Std Dev</b>	<b>1.5</b>
<b>Overall %CV</b>	<b>11%</b>

Values expressed as RP

# Validation Data – Linearity





# Link to clinical studies

Antigen #	Clinical Study #	Study Goals	ELISA Units used for assembly (per dose)
V00400602	359	Onset immunity	3867
	360	Duration of immunity	3867
	390	Onset immunity Ph. Eur.**	2956
0811689*	542	Short term study**	4844
V00400602	202	Safety 2x overdose pregnant Cows	6117
0811517	005	Safety overdose studies min age	Not determined (method not available, no archive material)

\* Assessed for ELISA using V00400602 as reference

\*\*Minimum age animals

- In 2002, antigen lot # 08115702 was assigned 2400 RU/ml and used as reference for in-process assay (R&D use only).
- In 2005, antigen lot # V00400602 was qualified as new antigen reference at 5600 RU/ml
- Antigen V00400602 was used
  - in vaccine V00500602U formulated at **3867** RU /ds for studies 359 and 360.
  - in vaccine RD013-013 formulated at **2956** RU/ds for study 390
  - as reference for Parkville antigen 0811689 that was used in formulating vaccine BIG071024B at 4844 RU/ds for study 542
  - In vaccine 001403901 used in overdose safety study in pregnant cows – ELISA value at assembly: **6117** RU/ds

# Pros/Cons of the *In-vitro* Assay

- Faster release time
  - Hamster assay up to 4 months
  - ELISA 1-2 Days
- Cost
  - Hamster assay \$8000 per test in EU (\$1000 in US)
  - ELISA \$500
- Monitor Reference
- Total development cost: ~\$4M
  - 10 years

# Challenges with replacing *in-vitro* tests for *Leptospira* (Bovine)

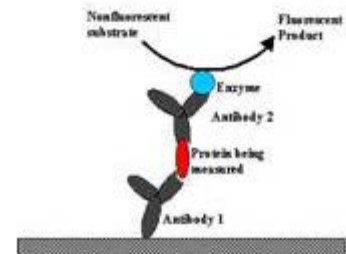
- Return on investment of resources
  - Cost vs benefit ratio in developing an alternative
    - 533 years to get return on R&D investment
      - Does not include product increase on shelf life (~ 4 weeks)
- Stringent Requirements on Parallelism (Full Curve)
  - Issue when comparing non-adjuvant antigen to adjuvant containing product
- Requirement of Reference
  - Requalification: High cost of studies (challenge model)
  - Reference Stability Monitoring Assay: Significant effort during product development

# Applying 3R's for Leptospira: A Case History

Time (Year)	Activity	Target "R"
1998-2000	Hamsters * (lethal endpoint)	----
1994-2010	Hamsters (bacterial culture)	Refine
2003-2006	Guinea pigs per monograph** (serology)	Refine
2005***-present	<i>In vitro</i> assessment of pre-formulated antigen (better prediction of potency)	Reduce
2010-present	<i>In vitro</i> assay for release	Replace



ELISA  
(Enzyme-Linked Immunosorbent Assay)



\*R&D evaluation, \*\*Validation failed, \*\*\*Monoclonal 6D12 in 2002

# Thank you!

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Srinivas Saginala

Mike Fisher

Dan Penka

Laurie Kopta

Angela Weber

Doug Gebhard

Paula Clare

Dick Kemmy

Martha Brown

Thierry Biot

Yvan Stukkens

Anne Thomas

Ruth McCabe

Concetta Salerno

Didier Thiry

David Gallo

Bonnie McMullen

Doug Drevo