

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

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International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward

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Introduction

- Background information on Spirovac[®] 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[®] *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

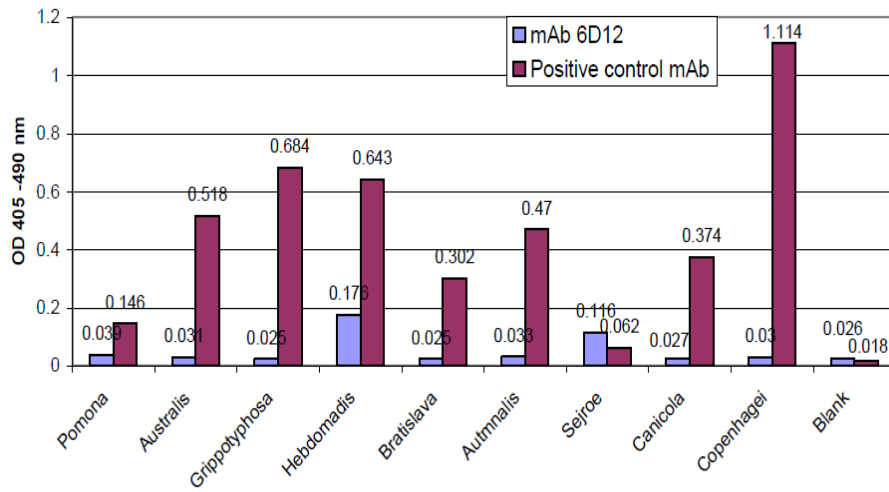


Reduce

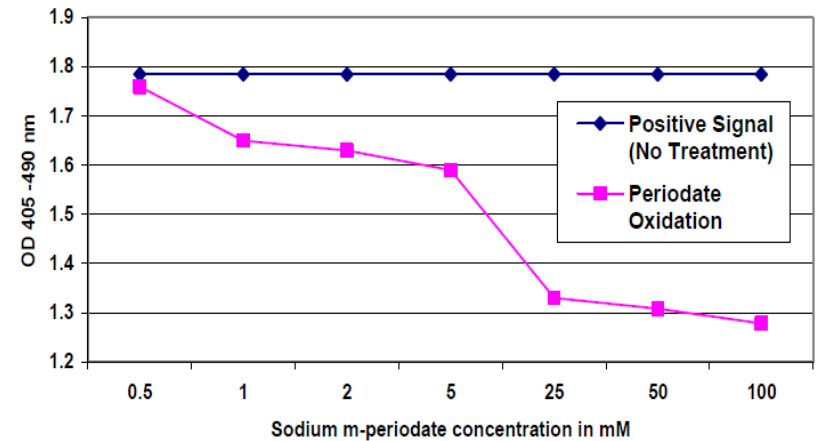
ELISA Reagents

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

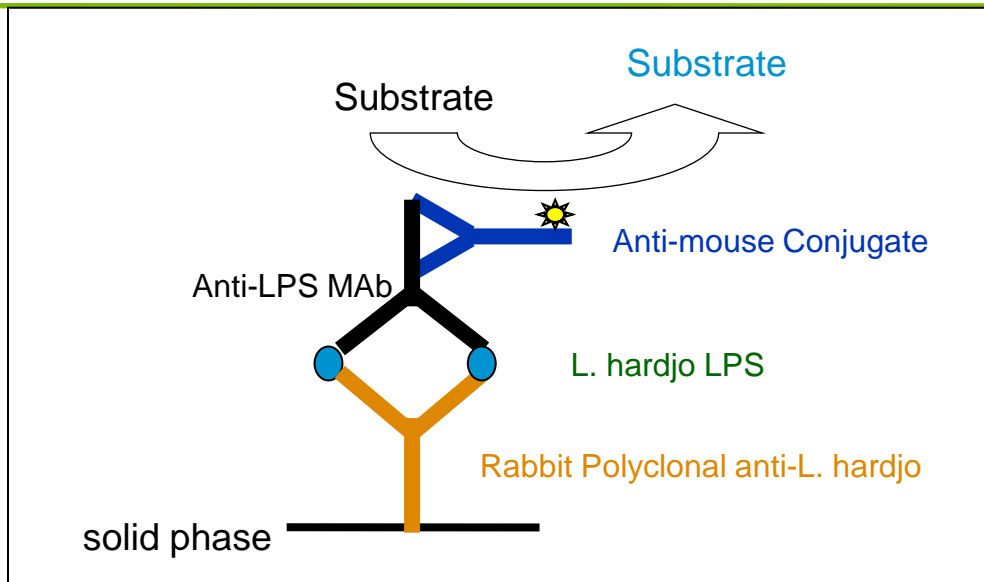
Anti *L. hardjo* mAb 6D12 Specificity in ELISA



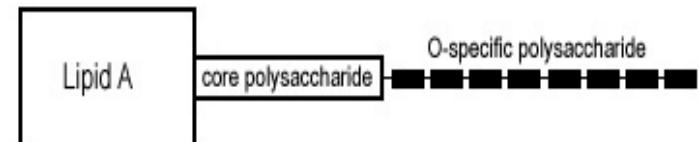
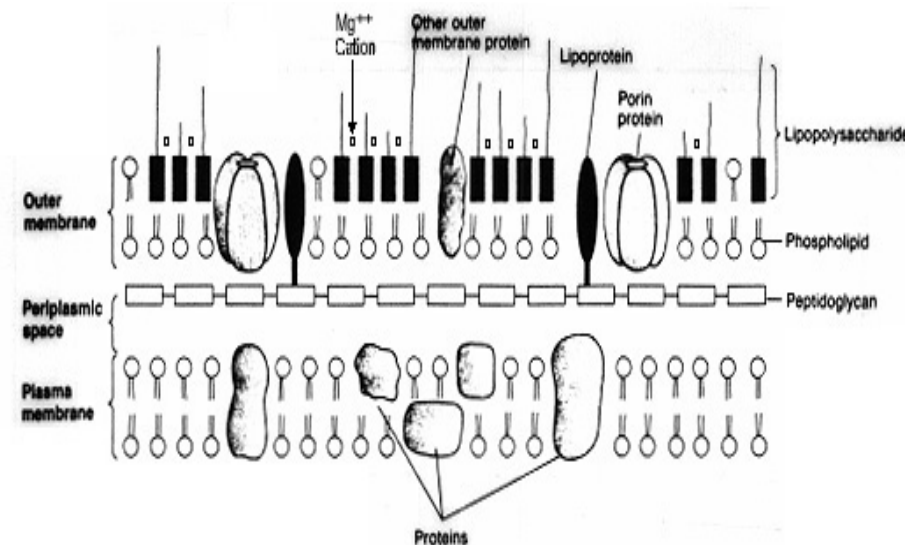
Anti *L. hardjo* mAb 6D12 - LPS Recativity -Sonicate Whole Cell



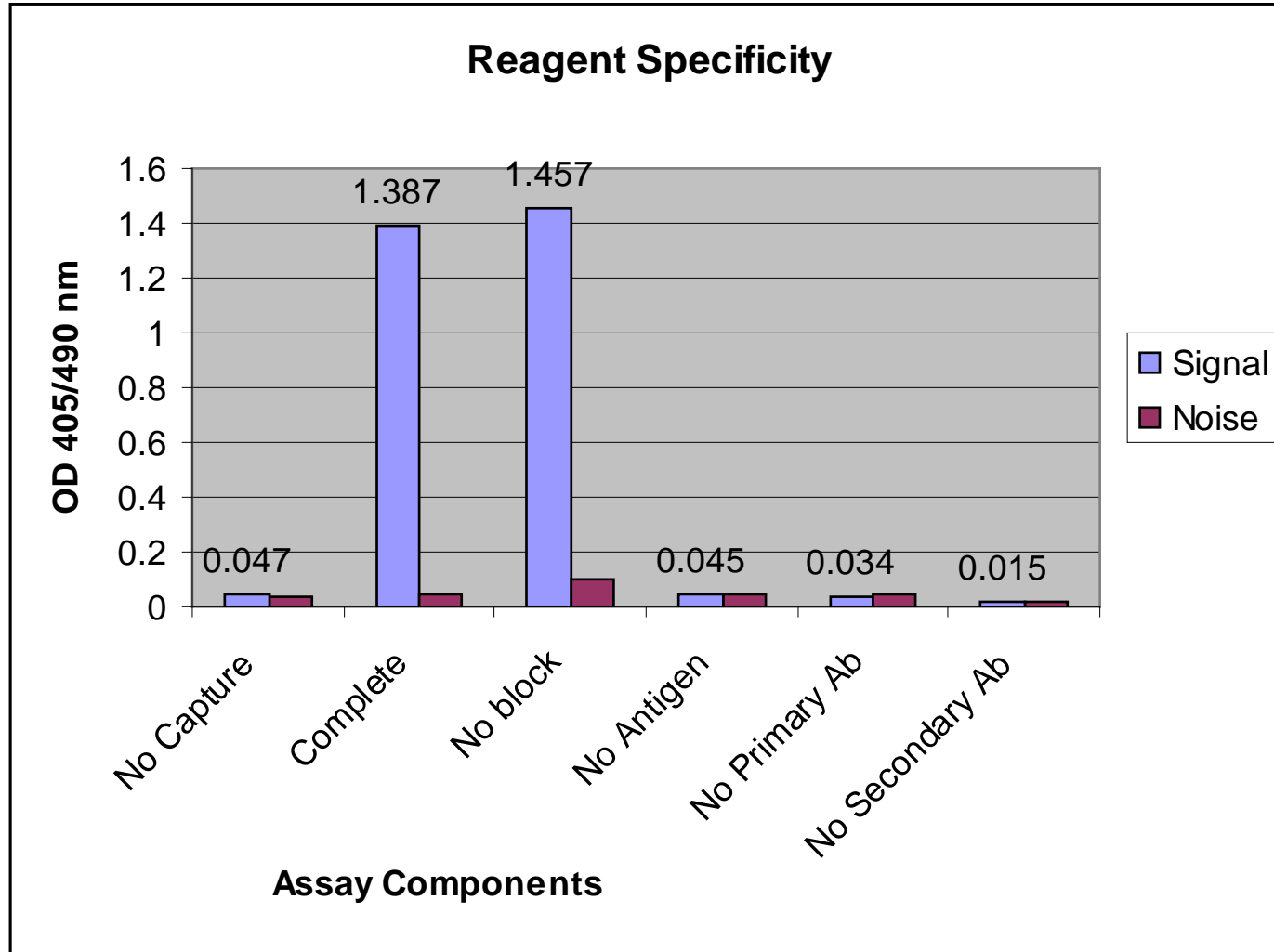
In Vitro ELISA for *L. borgpetersenii*, serovar hardjo LPS



- ◆ 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



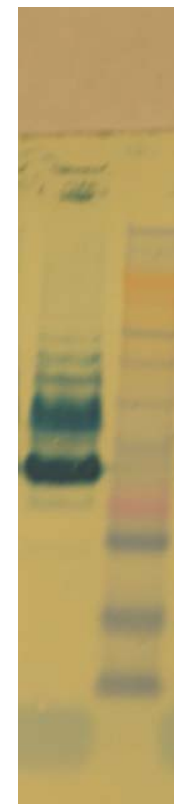
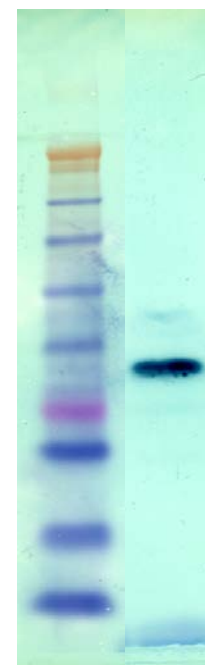
In process ELISA (Antigen Fluids) - Precision

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	
			Mean	7514	9834	690	2182
			STD	1214	1379	68	194
			%CV	16.16%	14.02%	9.88%	8.88%
					Overall %CV =	12.24%	

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

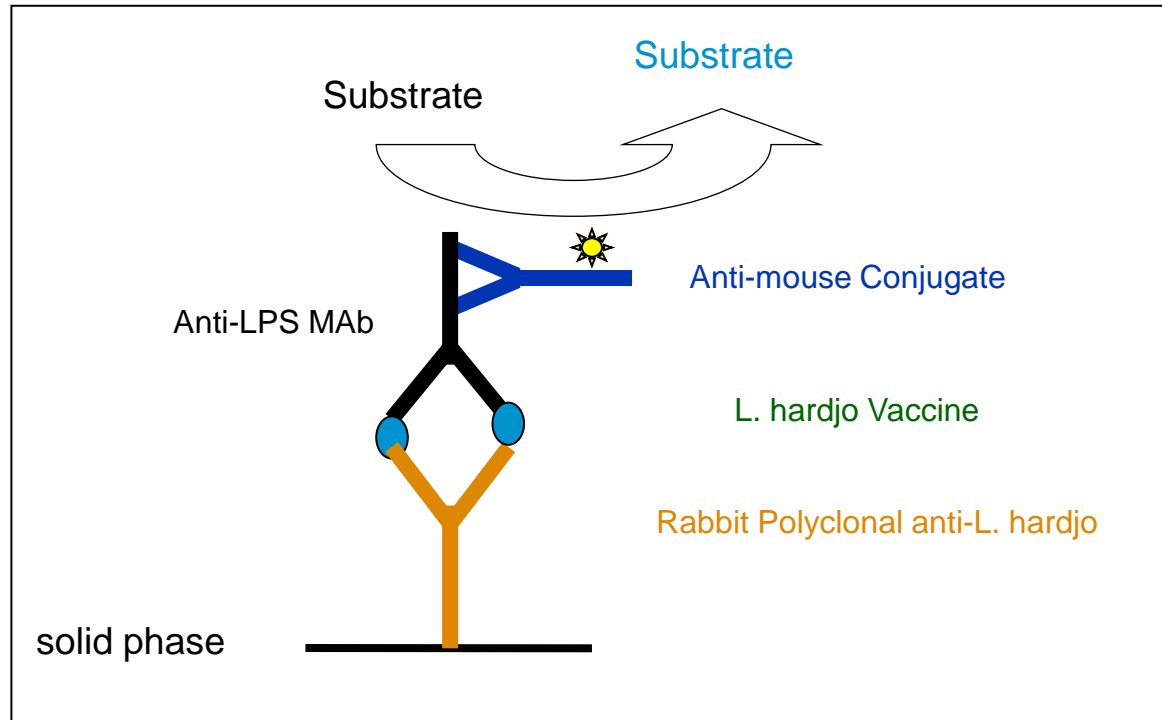
Lot A Lot B



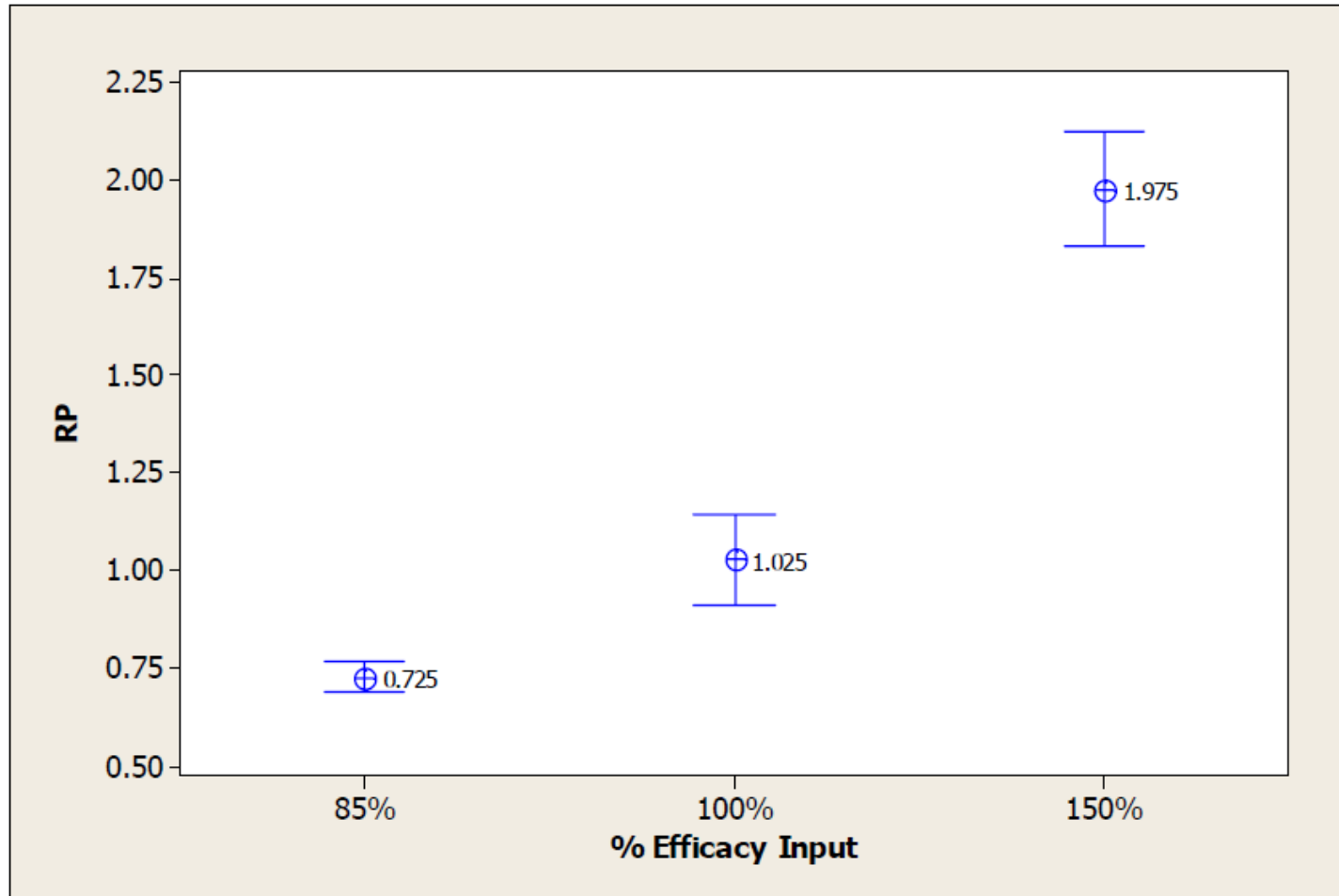
Reduce....

- 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

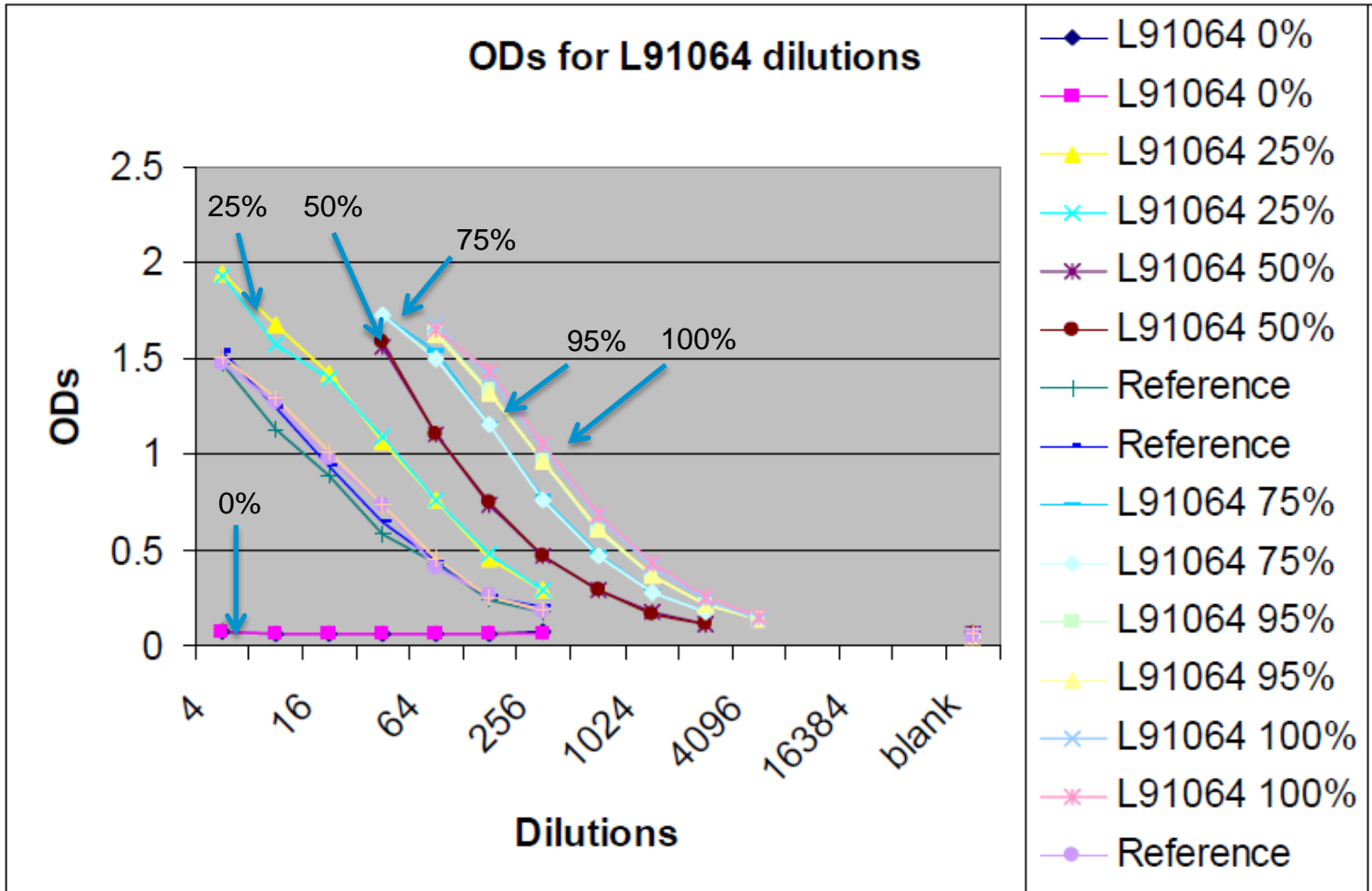
Final Product Potency ELISA – Replace....



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
Mean	0.7	1.0	1.9
%CV	0%	11%	5%
Technician 2	0.8	1.1	2.1
	0.7	0.9	1.7
	0.6	0.8	1.9
Mean	0.7	0.9	1.9
%CV	14%	15%	11%
Technician 3	0.7	0.9	1.8
	0.7	0.9	1.8
	0.7	0.8	1.7
Mean	0.7	0.9	1.7
%CV	0%	7%	3%
Overall mean	0.7	0.9	1.9
Overall Std Dev	0.1	0.1	0.1
Overall %CV	7%	13%	8%

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	Mean	10	Mean	7.5
	9	%CV	7%	%CV	18%
	10				
Mean	9				
%CV	10%				
Overall mean			8.8		
Overall Std Dev			1.4		
Overall %CV			16%		

Serial 2

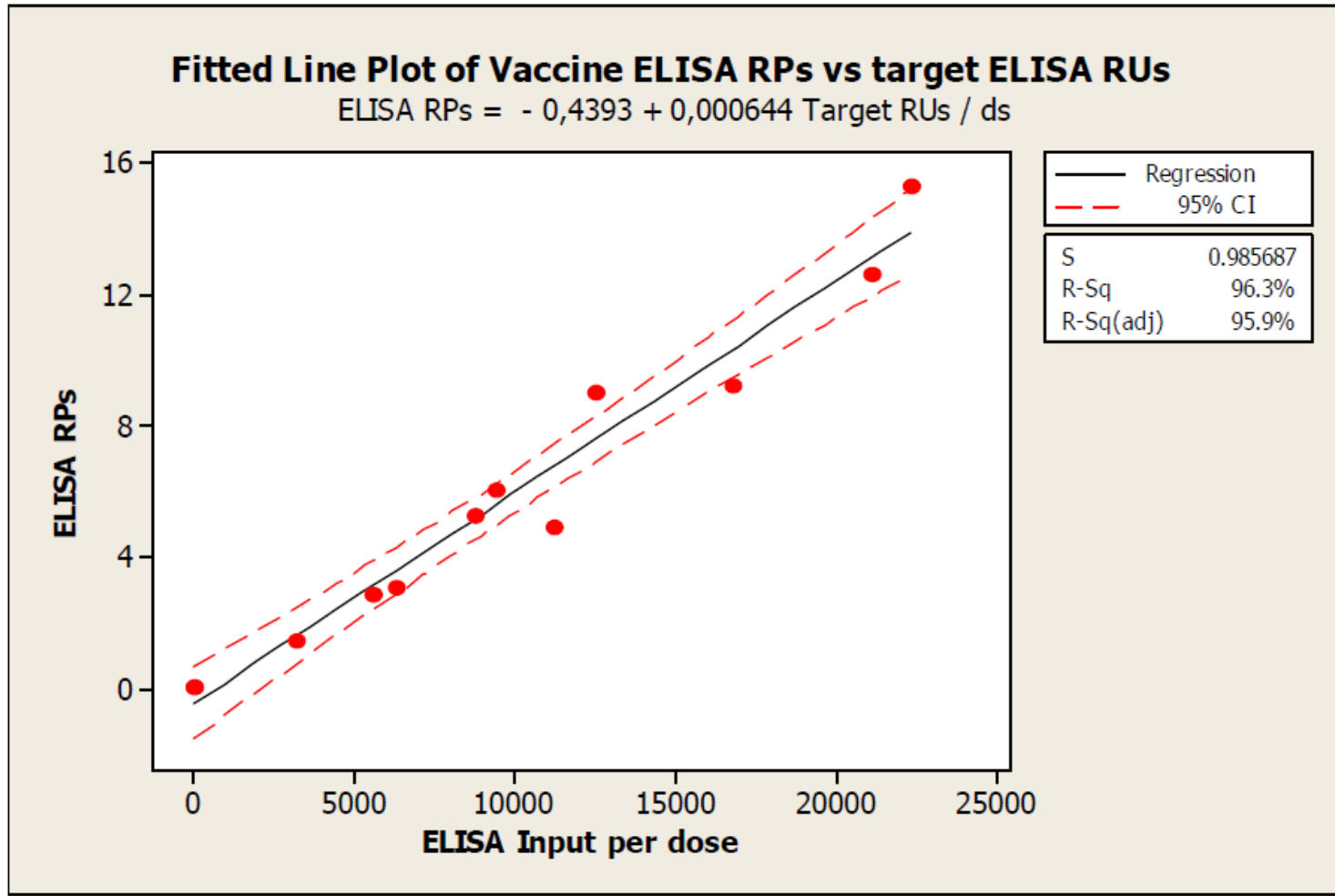
Overall mean	9.1
Overall Std Dev	1.6
Overall %CV	18%

Serial 3

Overall mean	14.1
Overall Std Dev	1.5
Overall %CV	11%

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)

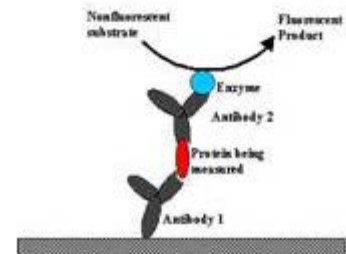
- Return on investment of resources
 - Cost vs benefit ratio in developing an alternative
 - 533 years to get return on R&D investment
 - Does not include product increase on shelf life (~ 4 weeks)
- Stringent Requirements on Parallelism (Full Curve)
 - Issue when comparing non-adjuvant antigen to adjuvant 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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