

Enzyme-Linked Immunosorbant Assays (ELISA) for the Detection of Antibodies
 to Pseudorabies Virus (Aujeszky's Disease)

John C. Lawrence
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Total Antibody

- PRV Screen Assay ■ Indirect Formats for Initial Screening of Large Numbers of Samples
- PRV Verification Assay ■ Verify Reactors Using PRV/NHC Test
- Non-Vaccinated Animals

Differential

- PRV gp1 (gE) Assay ■ Differentiation of gp1- Vaccinated and Field Infected Animals
- Can Also Be Used for Screening Non-Vaccinated Animals

————— Pseudorabies Test Formats —————

PRV Screen	96-Well Microtiter Assay (5 Plates/450 Tests)
PRV Verification	96-Well Microtiter Assay (2 Plates/84 Tests)
PRV gp1	96-Well Competitive Microtiter Assay 30 Plates (2,730 Tests) 6 Plates (Strips/456 Tests)

- Ready-to-Use Reagents
- Overnight Incubation Option with PRV gp1

————— PRV Test Protocols —————

	PRV Screen and Verification	PRV gp1
Sample Dilution	1:20	1:2
Sample	30' RT (100ul) 4x Wash	60' RT or 18-24 hrs @ 4°C (100 ul) 4x Wash
Conjugate	30' RT (100 ul) 4x Wash	20' RT (100 ul) 4x Wash
Substrate	15' RT (100 ul)	15' RT (100 ul)
Stop	100ul	50ul

- Read Absorbance @ 620nm/630nm/650nm

————— PRV Test Interpretations —————

	Indirect PRV Screen and Verification	Competitive/Blocking PRV gp1
Negative	S/P < 0.40	S/N > 0.70
Suspect		0.61 - 0.70
Positive	S/P ≥ 0.40	≤ 0.60

- Positives on Verification are Confirmed by S/NHC Ratio

$$\frac{OD \text{ Sample}}{OD \text{ NHC}} \geq 1.80 = \oplus$$

Sensitivity (Seroconversion/Temporal Series)

Experimentally Infected Animals
 Followed Over Time

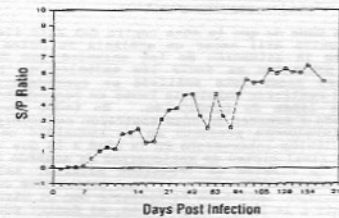
————— Sensitivity - Temporal Series —————

Pig #343 - Shope Strain

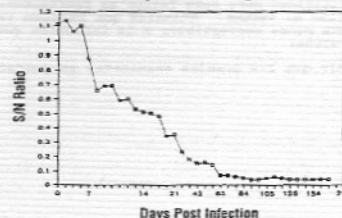
DPI	PRV Screen S/P	PRV gp1 S/N
0	.03 -	1.23 -
4	.07 -	1.03 -
5	-.06 -	1.18 -
6	.05 -	1.17 -
7	.20 -	1.00 -
8	.72 +	6.72 +
9	1.33 +	.56 +
10	2.28 +	.50 +
11	2.70 +	.47 +
12	3.41 +	.39 +
13	3.78 +	.37 +
14	3.87 +	.35 +
15	4.32 +	.33 +
16	4.14 +	.27 +
18	4.43 +	.20 +
21	3.32 +	.19 +
28	4.8 +	.15 +

————— Sensitivity —————

PRV ELISA
 Shope Strain: Pig 343



PRV-gp1 ELISA
 Shope Strain: Pig 343



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(73) *antibody to pseudorabies virus*

— Sensitivity - Temporal Series —

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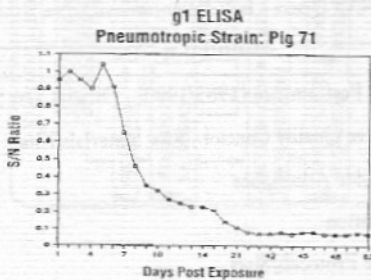
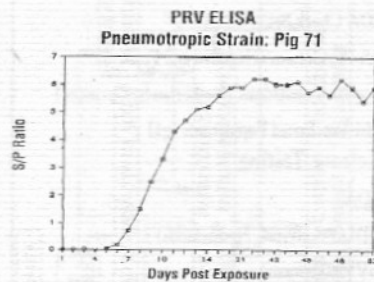
Pig #71 - Pneumotropic Strain

DPI	PRV Screen S/P	PRV gp1 S/N
0	0 -	1.00 -
1	0 -	0.95 -
2	0 -	1.00 -
3	0 -	0.95 -
4	0 -	0.90 -
5	.03 -	1.04 -
6	.19 -	0.91 -
7	.73 +	0.65 +
8	1.50 +	0.46 +
9	2.50 +	0.35 +
10	3.31 +	0.32 +
11	4.37 +	0.27 +
12	4.71 +	0.25 +
13	5.10 +	0.23 +
14	5.60 +	0.23 +
16	5.90 +	0.21 +
18	5.93 +	0.14 +
21	6.22 +	0.11 +
28		0.08 +

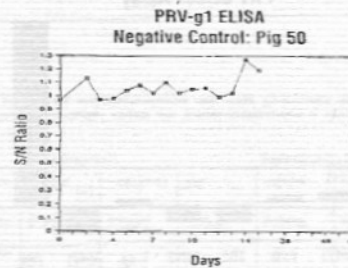
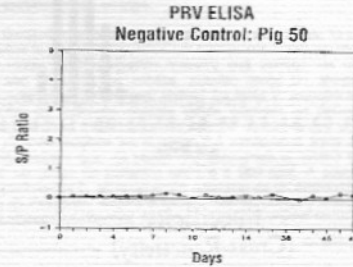
Control Pig #50

DPI	PRV Screen S/P	PRV gp1 S/N
0	.02	.97
1	.04	NT
2	.04	1.13
3	.05	.97
4	.05	.98
5	.06	1.04
6	.07	1.08
7	.10	1.02
8	.16	1.10
9	.12	1.02
10	.01	1.05
11	.12	1.06
12	.03	.99
13	.06	1.02
14	.10	1.27
22	.03	1.19

— Sensitivity —



— Sensitivity —



— Specificity —

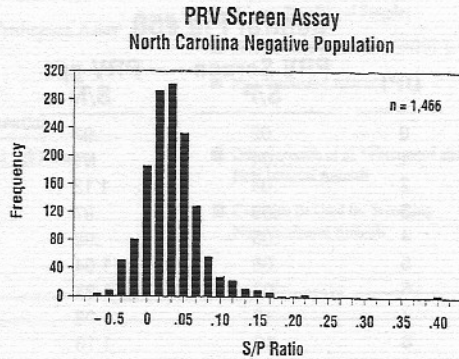
■ Negative Populations

■ Cross-Reactivity

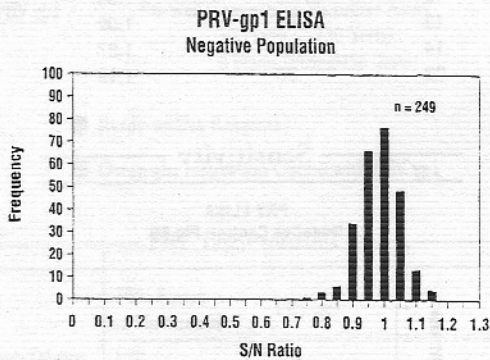
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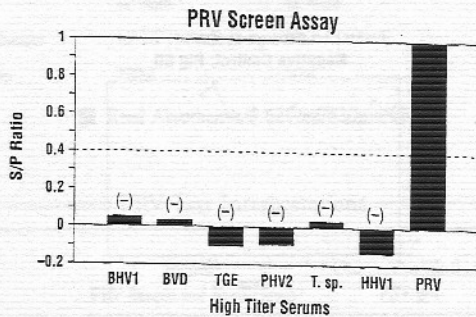
Specificity



Specificity



Specificity
 (Cross-Reactivity)



■ PRV Antigen used in PRV (S), PRV (V) and PRV-gp1 is Identical

Reproducibility

■ Plate CV

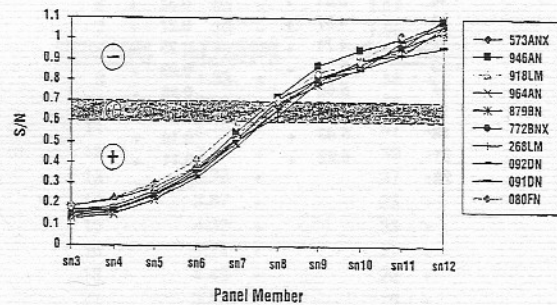
■ Lot-to-Lot

Plate Coefficient of Variation (CV)

	Lot	Plate (CV)
1995	414	5.2%
	142	4.9%
	003	3.3%
	488	6.0%

1996	950	4.0%
	279	4.8%
	035	4.5%

■ Plate Batches are Tested Throughout the Entire Plate Run
 — ADV gp1 Sensitivity Panel —



Quality Control

Region Specific Samples

- USDA Check Set
 - USA Populations (Negative and Positive)
 - European Populations (Netherlands, Germany)
 - Multi-Vaccinated Populations (g1)
- Lot Release Testing
- USDA
 - ID-DLO (Lelystad, Netherlands)
 - BGVV (Germany)
 - CNEVA (France - Includes International Serum Standards)

Summary

- Proven Performance (1985/1990) - Official Test Status
- Extensive Quality Control - Raw Materials/Kits
- Worldwide Acceptance
- Automation
- Flexible Protocols/Sizes
- Technical Support
- Constant Improvements to Maintain Market Relevance