BVD TESTING LABORATORY

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CST VETERINARY LABORATORY

BVDV ELISA TESTS

New Research Delivers Reliable, Accurate Alternative ELISA

Antigen Capture ELISA (ACE) tests provide the greatest accuracy for BVDV PI detection.

- Multiple studies have shown that the E^{rns} based ELISA test provide superior accuracy over other testing methods such as IHC and PCR [1,2,3].

Previous studies have shown that other ELISA's such as NS3 (p80) based tests have lacked the sensitivity of E^{rns} based ELISA's[4]

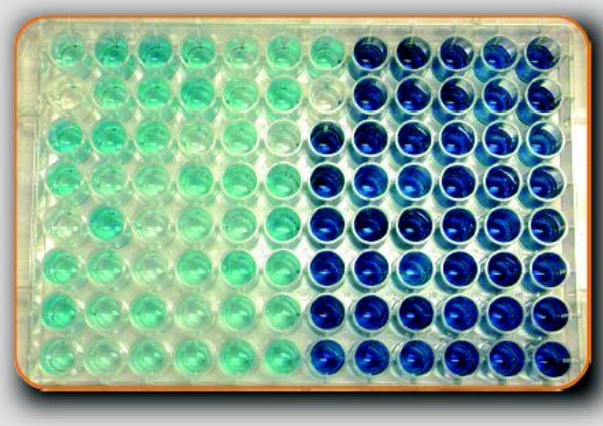
- These studies concluded that the NS3 protein in BVDV PI animals lack stability, solubility and have too varied concentration to allow accurate detection.
- THIS WAS CORRECT THEN, BUT NOT NOW.

New research resolves issues of stability, variability and solubility of NS3 protein

- A new, revolutionary extraction buffer, BVDView™ provides stability to the NS3 protein. It increases solubility and reduces the variability of the protein, making NS3 ACE detection accurate, providing an equally reliable and more economical alternative.

BVDView™ Improves NS3 Sensitivity

Inadequate solubility and variability in concentration of the NS3 protein has made BVDV PI detection difficult with alternative ACE tests and lead to many false conclusions about quantity and detectability of NS3 protein. BVDView™ has eliminated this problem, making NS3 ELISA a reliable, accurate, cost effective option.



Picture shows increase in signal from solubilization of NS3 protein with BVDView™. Left half of plate used routine PBS extraction and right half used BVDView™ extraction on the same samples.

NS3 ACE Solubility and Variability study

Positive samples from 178 adult animals initially identified by a commercial E^{rns} kit and confirmed by IHC and PCR tests were utilized in this study. These samples returned the lowest optical density (OD) signals by a commercial NS3 ACE kit [7]. In addition one sample known to test negative by a commercial E^{rns} kit was also used. Samples were tested with a commercial NS3 ACE kit [7] according to a package insert, by CST's NS3 test using BVDView extraction buffer and by a commercial E^{rns} ACE test according to a kit protocol.

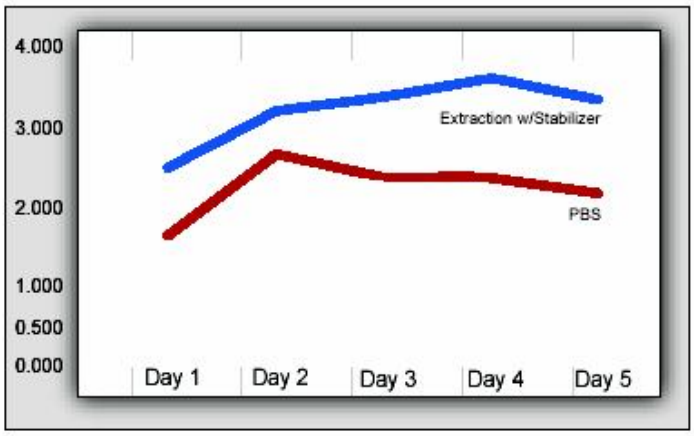
	NS3 ACE [7]	CST ACE	E^{rns} ACE [9]
Avg OD	1.039	3.517	1.169
OD Range	0.065 - 3.314	1.049-4.138	0.342-1.883
OD Variation	38%	10%	9%

The use of BVDView™ with a NS3 capture ELISA not only greatly enhances BVDV PI detection signal but also removes the variability of NS3 protein levels. BVDView™ increases signal strength more than 400% and reduces variability almost 300%. The use of BVDView™ with a NS3 ELISA provides a much superior signal to the E^{rns} test. [8]

NS3 PROTEIN STABILITY

New BVDView™ extraction buffer provides unparalleled protein stabilization.

Extraction w/Stabilizer vs PBS Extraction Stability at 95°F



Thirteen confirmed positive samples were evaluated in this study. Average optical density of the samples are shown when tested in extraction buffer with stabilizer and normal PBS extraction. Stabilizer provides 52% greater baseline signal and continued to increase in signal over the 120 hour study. PBS extraction showed lower signals, protein instability and decreasing signal during the study.

All samples in BVDView™ resulted in positive outcomes at all times. Eleven tests resulted in false negative outcomes with routine PBS extraction.

CST ACE Performance Study With BVDView™

New BVDView™ extraction buffer increases performance of NS3 ACE to equal or better than competing technologies

	NS3 ACE [10]	Commerical NS3 ACE [7]	CST ACE w/ BVDView™	Commercial E^{rns} ACE [9]
Sensitivity	95.5% (105/110)	95.5% (171/179)	100% (179/179)	99.4% (178/179)
Specificity	100% (93/93)	100% (152/152)	99.9% (2916/2917)	99.9% [5] (21654/21657)

The Commercial NS3 and E^{rns} Antigen Capture ELISA (ACE) tests were conducted as per package inserts. The Central States Testing NS3 ACE test was conducted according to the in-house protocol and utilized BVDView™ extraction buffer.

Study shows the CST NS3 Antigen Capture ELISA test has **superior** sensitivity to the E^{rns} ACE and other NS3 ACE. Specificity study shows no difference between ELISA tests.

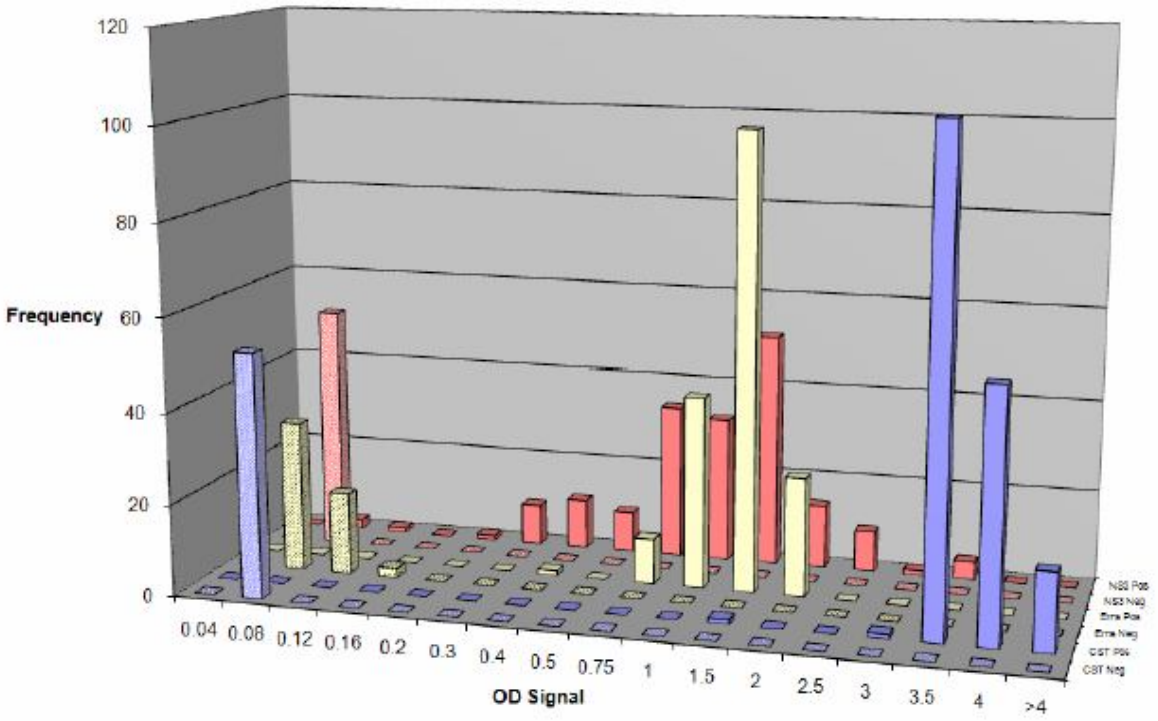
References

1. Edmondson MA, Givens MD, Walz PH, et al. Comparison of tests for detection of bovine viral diarrhea virus in diagnostic samples. J Vet Diagn Invest. 2007;19.
2. Ridpath JF, Hessman BE, Neill JD, et al. Parameters of ear notch samples for BVDV testing: Stability, size requirements and viral load. Proceedings from AABP Convention. September 2006; St Paul Minnesota.
3. Fulton RW, Hessman BE, Ridpath JF, et al. Utilization of Multiple Diagnostic Tests to Identify Cattle with Bovine Viral Diarrhea Virus and Duration of Positive Tests in Persistently Infected Cattle. Accepted for publication, Can J Vet Res 2008.
4. Fux, RG. Development and evaluation of diagnostic methods for detecting bovine viral diarrhea virus in dried ear biopsy samples using antigen-ELISAs and real time RT-PCR (dissertation). Munich, Germany: University of Munich; 2007.
5. Fulton RW, Hessman B, Johnson BJ, et al. Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b and 2a in persistently infected cattle entering the feedlot. J Am Vet Med Assoc 2006;228:578-584.
6. Veridia LLC, Brookfield, WI
7. Prionics PrioCHECK BVDV Ag, Zurich, Switzerland
8. Data on file. Central States Testing LLC, Sublette, Ks.
9. IDEXX HerdCheck, Westbrook, Maine, USA
10. Data from IDEXX marketing brochure

CST has contributed to the development of BVDView™ technology.

Comparison of two different ACE kits and a NS3 ELISA test using BVDView™

Histogram of OD Signals comparing NS3, Erns & CST NS3 ELISA Tests on 178 Confirmed Positives and 53 Negatives



NS3 NEG | NS3 POS | E^{rns} NEG | E^{rns} POS | CST NEG | CST POS

	Commercial NS3 ACE[1]	Commercial E^{rns} ACE[2]	CST ACE w/BVDView™ [3]
Sensitivity	95.5%	99.4%	100%
Specificity	100%	99.9%	99.9%
Average OD (POS)	1.039	1.169	3.517
OD Range (POS)	0.065-3.314	0.342-1.883	1.049-4.138
OD Variation (POS)	38%	9%	10%
Standard Deviation (POS)	+/- 0.62	+/- 0.29	+/- 0.32
Separation (POS-NEG)	-0.003	0.215	0.981

CST test w/BVDView™ provides:

- greater sensitivity
- greater average OD signals of positives
- lower signal of negatives than E^{rns} ELISA
- similar variation to E^{rns} ELISA
- four times greater separation between positives and negatives

References:

1. Prionics PrioChek BVDV Ag, Zurich, Switzerland
2. IDEXX Laboratories, Westbrook, ME
3. Veridia LLC, Brookfield, WI