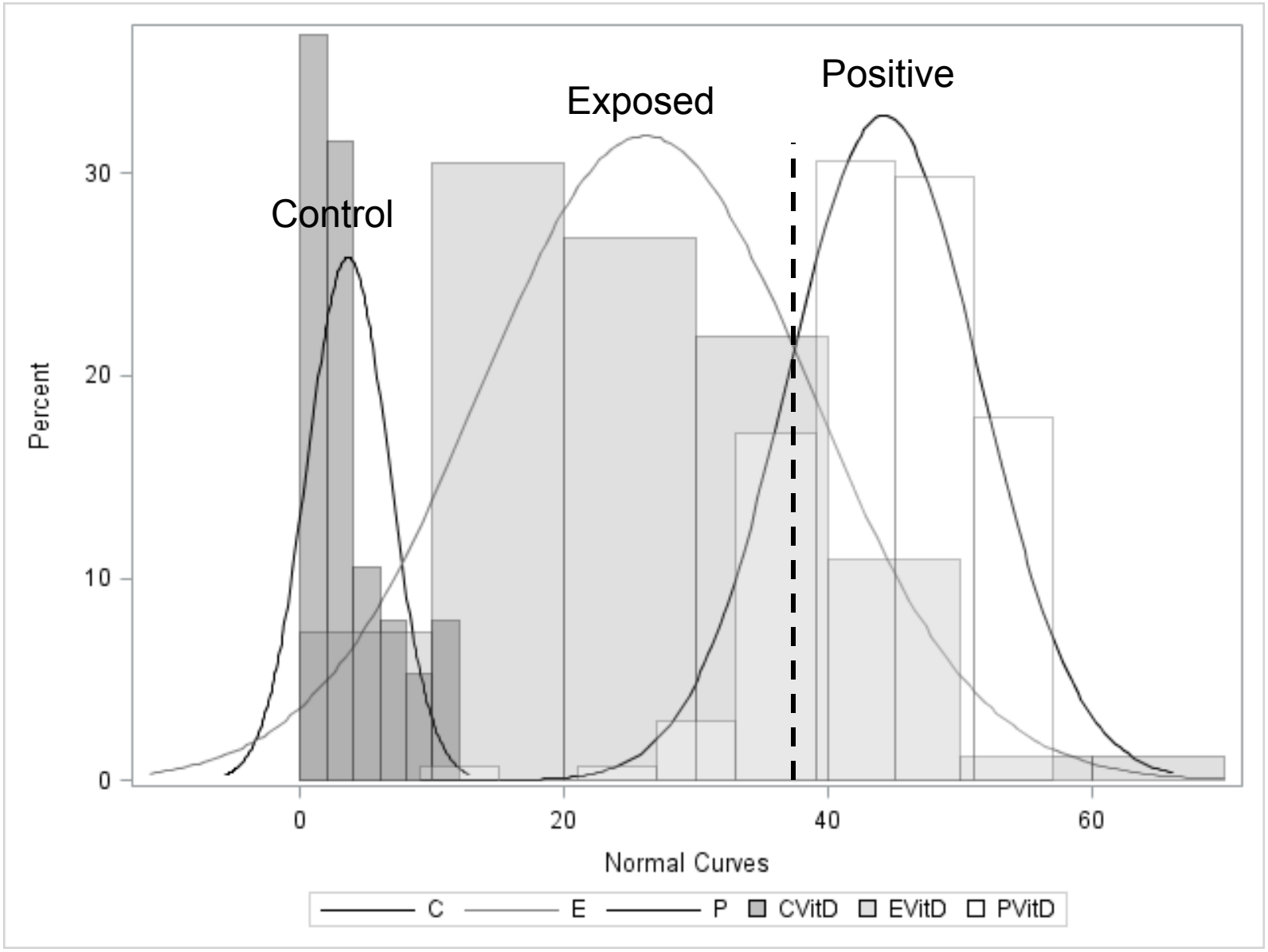
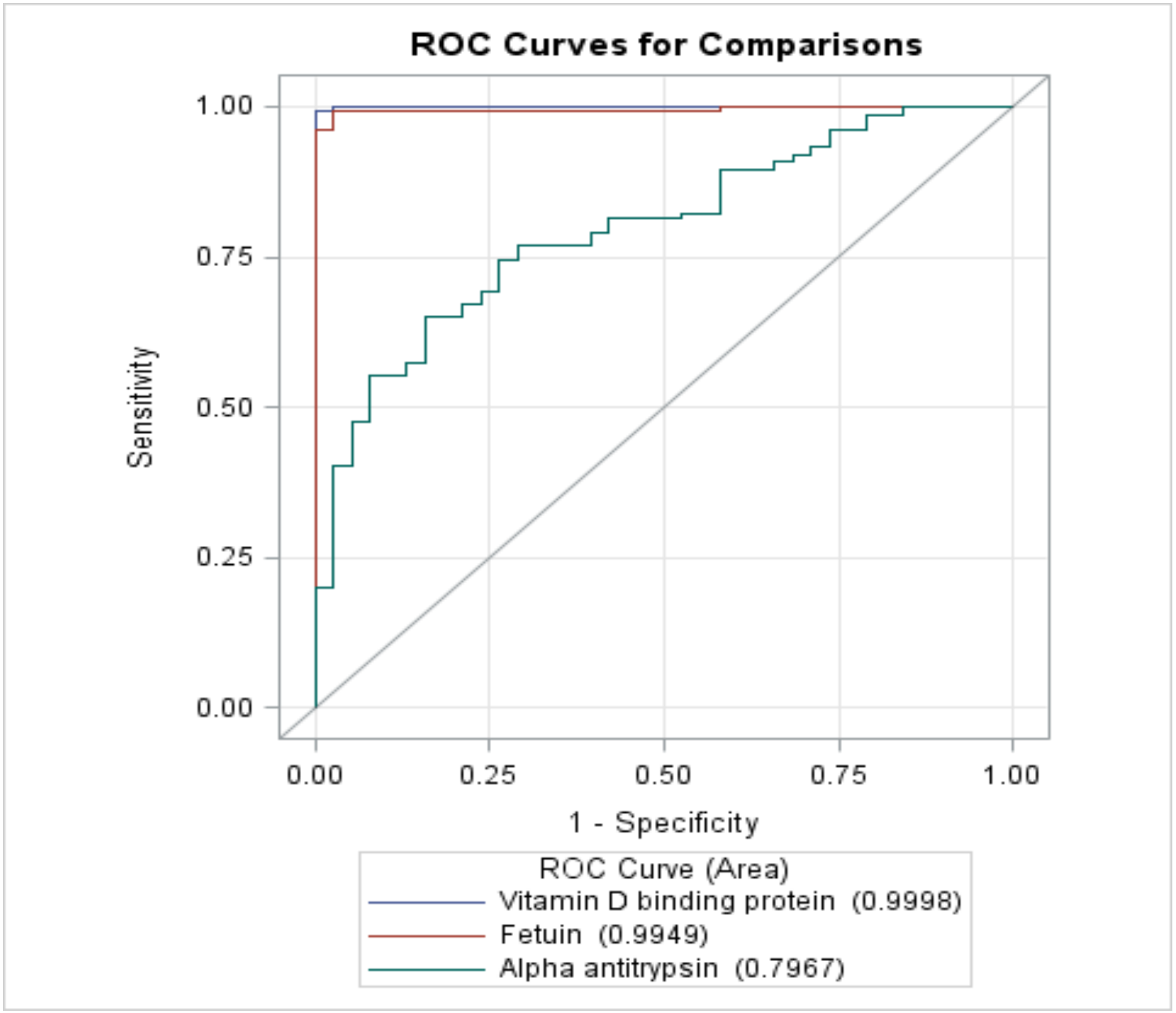
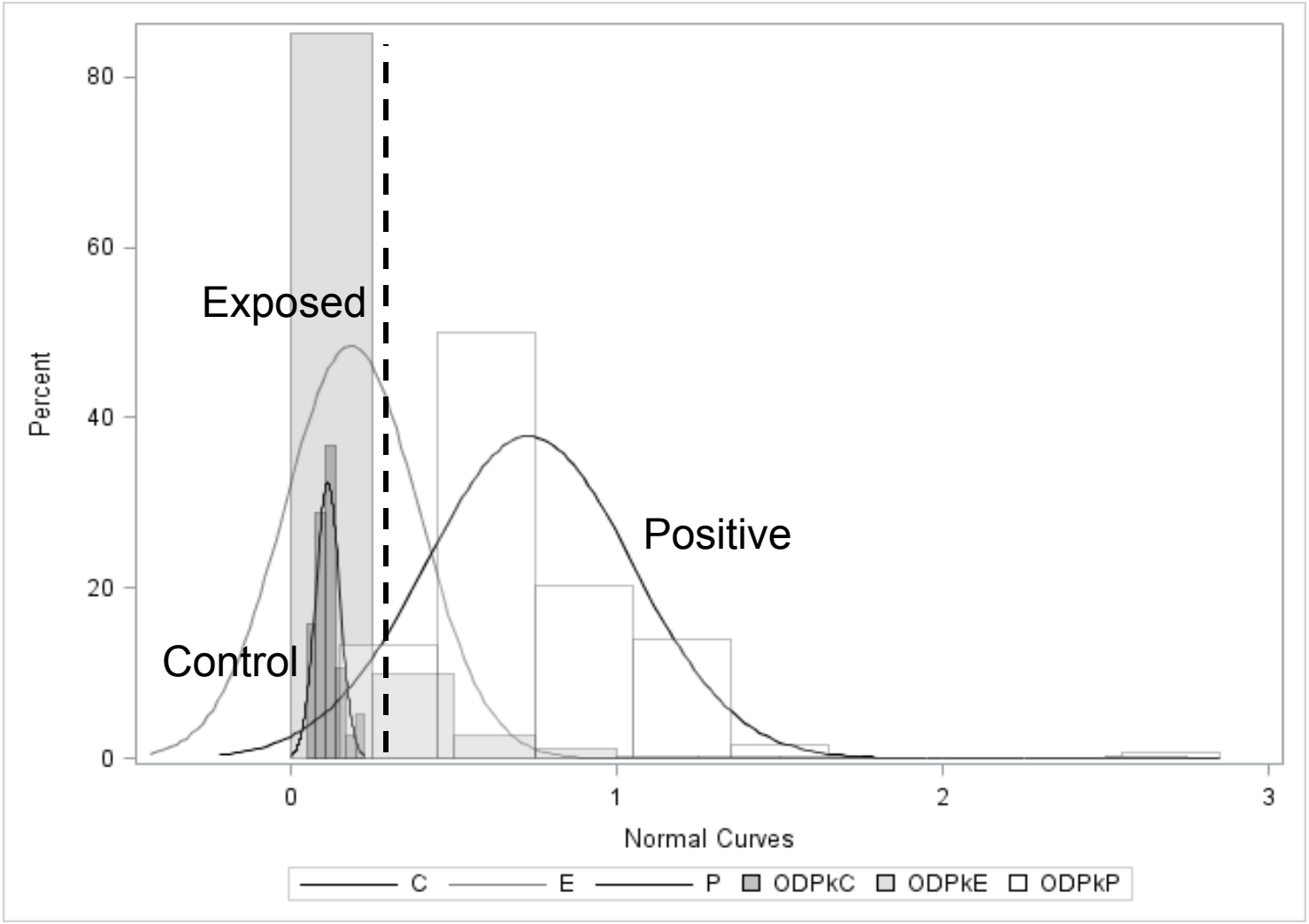
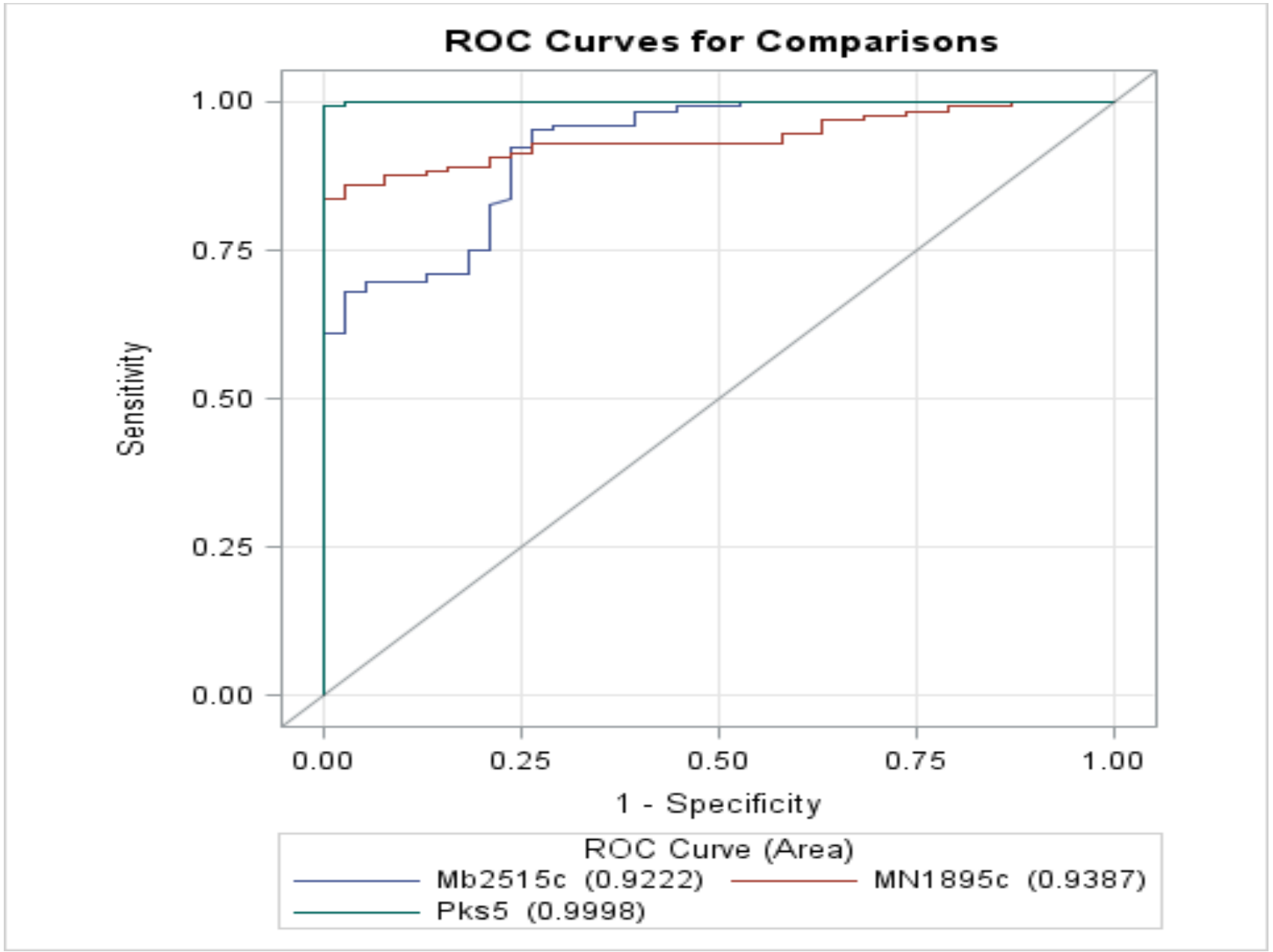


Vitamin D Binding Protein


ROC Curves for Comparisons
Sensitivity
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
1 - Specificity
ROC Curve (Area)
Vitamin D binding protein (0.9998)
Fetuin (0.9949)
Alpha antitrypsin (0.7967)


Exposed
Positive
Control
Percent
80
60
40
20
0
0
1
2
3
Normal Curves
C
E
P
ODPkC
ODPkE
ODPkP

Pks5


ROC Curves for Comparisons
1.00
0.75
0.50
0.25
0.00
Sensitivity
0.00
0.25
0.50
0.75
1.00
1 - Specificity
ROC Curve (Area)
Mb2515c (0.9222)
MN1895c (0.9387)
Pks5 (0.9998)

Pks5 Cutoffs Based on Sensitivity and Specificity

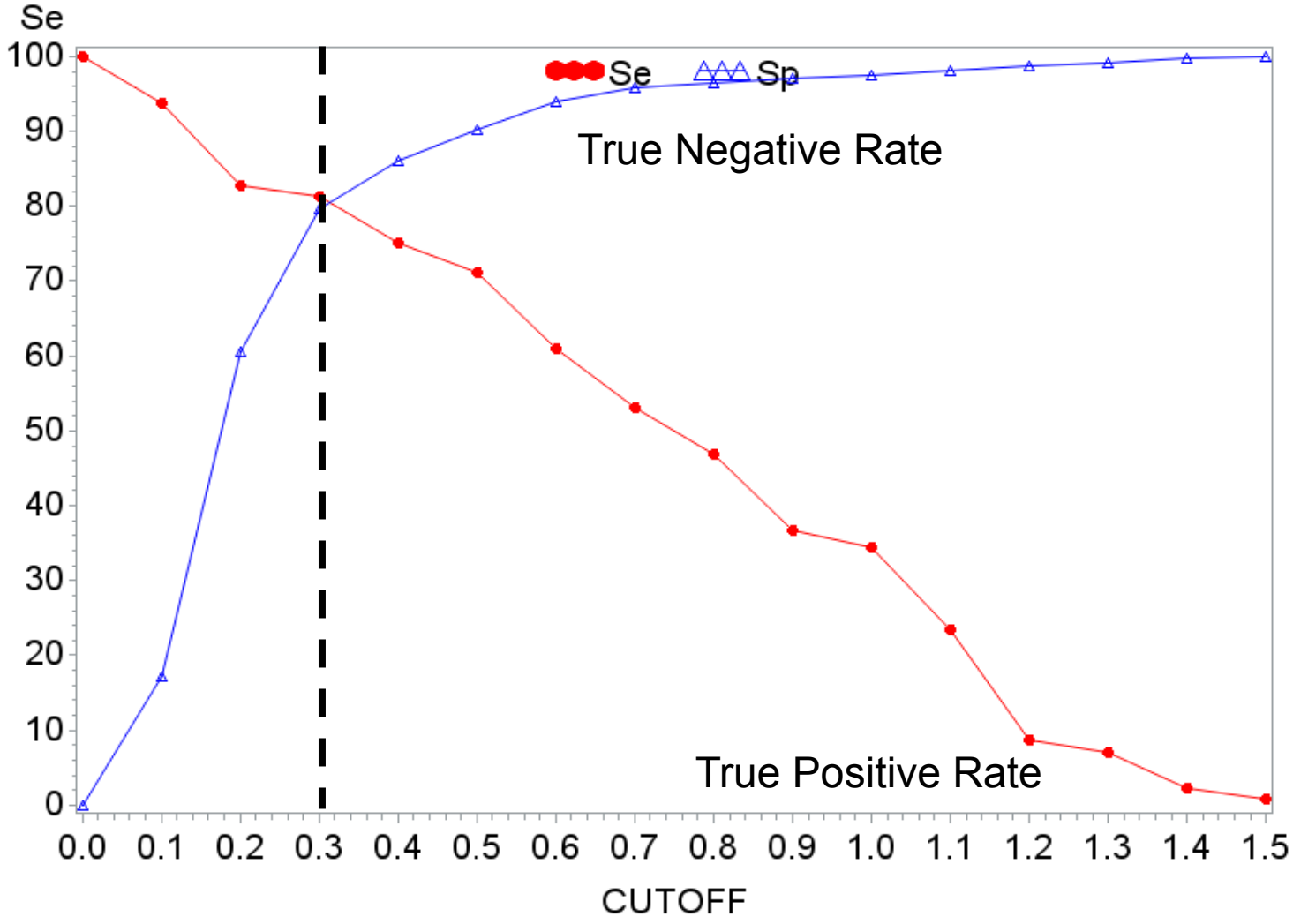
Obs	CUTOFF	TEST	Status	COUNT	PERCENT	PCT_ROW	TP_RATE	FP_RATE	Se	Sp
1	0.0	R	C	38	22.8916	22.892	100.000	100.000	100.000	0.000
2	0.1	R	C	23	13.8554	15.232	100.000	60.526	100.000	39.474
3	**0.2**	**R**	**C**	**2**	**1.2048**	**1.538**	**100.000**	**5.263**	**100.000**	**94.737**
4	**0.3**	**R**	**P**	**127**	**76.5060**	**100.000**	**99.219**	**0.000**	**99.219**	**100.000**
5	**0.4**	**R**	**P**	**118**	**71.0843**	**100.000**	**92.188**	**0.000**	**92.188**	**100.000**
6	0.5	R	P	101	60.8434	100.000	78.906	0.000	78.906	100.000

Obs = Number of observations
TP_Rate = True Positive Rate
FP_Rate = False Positive Rate
Se = Sensitivity
Sp = Specificity

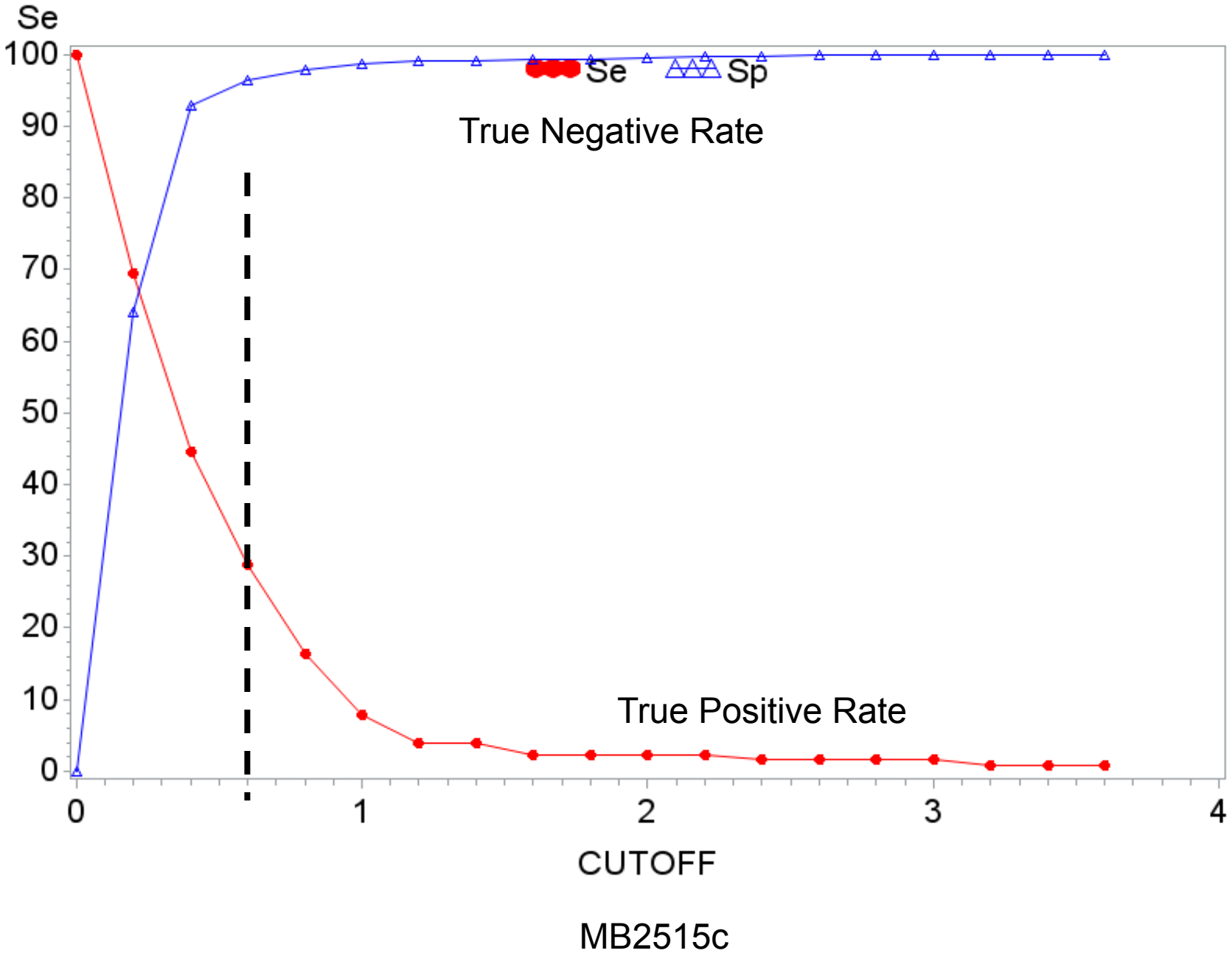
Pks5 Cutoffs Based on Sensitivity and Specificity

Obs	CUTOFF	TEST	Status	COUNT	PERCENT	PCT_ROW	TP_RATE	FP_RATE	Se	Sp
1	0.0	R	E	424	76.8116	76.8116	100.000	100.000	100.000	0.0000
2	0.1	R	E	311	56.3406	70.8428	100.000	73.349	100.000	26.6509
3	**0.2**	**R**	**E**	**104**	**18.8406**	**44.8276**	**100.000**	**24.528**	**100.000**	**75.4717**
4	**0.3**	**R**	**E**	**42**	**7.6087**	**24.8521**	**99.219**	**9.906**	**99.219**	**90.0943**
5	**0.4**	**R**	**E**	**29**	**5.2536**	**19.7279**	**92.188**	**6.840**	**92.188**	**93.1604**
6	**0.5**	**R**	**E**	**21**	**3.8043**	**17.2131**	**78.906**	**4.953**	**78.906**	**95.0472**
7	0.6	R	E	13	2.3551	14.7727	58.594	3.066	58.594	96.9340
8	0.7	R	E	10	1.8116	14.9254	44.531	2.358	44.531	97.6415
9	0.8	R	E	8	1.4493	17.0213	30.469	1.887	30.469	98.1132
10	0.9	R	E	6	1.0870	17.1429	22.656	1.415	22.656	98.5849
11	1.0	R	E	4	0.7246	15.3846	17.188	0.943	17.188	99.0566
12	1.1	R	E	4	0.7246	20.0000	12.500	0.943	12.500	99.0566
13	1.2	R	E	3	0.5435	25.0000	7.031	0.708	7.031	99.2925
14	1.3	R	E	3	0.5435	37.5000	3.906	0.708	3.906	99.2925

Obs = Number of observations
TP_Rate = True Positive Rate
FP_Rate = False Positive Rate
Se = Sensitivity
Sp = Specificity


Se
100
90
80
70
60
50
40
30
20
10
0
Se
Sp
True Negative Rate
True Positive Rate
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
CUTOFF

MB1895c


Se
100
90
80
70
60
50
40
30
20
10
0
Se
Sp
True Negative Rate
True Positive Rate
0
1
2
3
4
CUTOFF

MB2515c

Supplemental Figures:

Figure S1: Vitamin D binding protein (VDBP) histogram.

Relative densities were collected from negative control (n=38), negative bTB exposed (n = 82) and infected (n = 128) animals. Cutoff value (38.50) was determined based on ROC analysis and true negative rate vs. true positive rate. Control, positive and exposed groups showed clear separation.

Figure S2: Receiver Operating Characteristic (ROC) curves for host biomarkers.

Each point on ROC curves is the fraction of bTB positive cattle (true-positive rate) versus the corresponding fraction of negative controls (false-positive rate). VDBP and fetuin-A are the most reliable biomarkers (AUC = 0.9998 and 0.9949, respectively) and alpha-1 antitrypsin is a moderately reliable biomarker (AUC = 0.7967). VDBP = blue line, fetuin-A = red line, and alpha-1 antitrypsin = green line.

Figure S3: Polyketide synthetase 5 (Pks5) histogram.

Relative densities were collected from negative controls (n = 38), negative bTB exposed (n = 428) and infected (n = 128) animals. Cutoff value (0.4 nm) was determined based on ROC analysis and true negative rate vs. true positive rate.

Figure S4: Receiver Operating Characteristic (ROC) curves for pathogen biomarkers.

Each point on ROC curves is the fraction of bTB positive cattle (true-positive rate) versus the corresponding fraction of negative controls (false-positive rate). Pks5, MB2515c, and

MB1895c are all reliable biomarkers (AUC = 0.9998, 0.9222, and 0.9387, respectively). Pks5 = green line, MB1895c = red line, and MB2515c = blue line.

Figure S5: Pks5 cutoffs based on sensitivity and specificity.
The true negative rate and true positive rate using Pks5 as a biomarker were plotted against each other. Cutoff values ranging from 0.2-0.5 nm may be used in field applications dependent upon bTB prevalence.

Figure S6: Pks5 cutoffs based on sensitivity and specificity comparing negative controls and bTB infected animals.
The true negative rate and true positive rate using Pks5 as a biomarker were plotted against each other. Cutoff values ranging from 0.2-0.4 nm provided maximum sensitivity and specificity.

Figure S7: MB1895c distinguished bovine tuberculosis positive and negative exposed animals. The true negative rate and true positive rate using MB1895c as a biomarker were plotted against each other. The optimal cutoff value corresponded to 0.3 nm. Cutoff value corresponds to a sensitivity of 81.250% and specificity of 79.717%.

Figure S8: MB2515c is a moderately reliable biomarker for bovine tuberculosis.
The true negative rate and true positive rate using MB2515c as a biomarker were plotted against each other. The optimal cutoff value corresponded to 0.2 nm. Cutoff value corresponds to a sensitivity of 69.531% and specificity of 64.151%