Veridex BLN Assay

Statistical Design and Analysis of Pivotal Study

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Outline

- Study Design
- Analysis, All subjects
- Analysis, Subjects with FS H&E
  - BLN assay & FS H&E compared
- Variability by Site
- Summary

Study Design

- **Cut-off Study (N=312)**

  CK19 ≤ 31   MG ≤ 30   IC ≤ 36

- **Pivotal Study (N = 421)**

  Primary Endpoints: $Se \geq 0.7$, $Sp \geq 0.9$

- Study designs were identical.
- Conducted at same investigational sites
- Transition to Pivotal study seamless.

Bayesian Interim Analysis Plan

Look every 50 subjects, N = 200-700.

- Stopping rules:
  If $Pr(Se \geq 0.7) > 0.985$ AND
  $Pr(Sp \geq 0.9) > 0.985$,
  then stop and declare success.

  If predictive probability of success < 0.05, then stop for futility.

- Prior distribution used was non-informative.

Reality: Only 1 interim look at N=413, Other amendments submitted.

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Types of Analyses

Sample Sizes	All Subjects	Subjects With FS H&E Results
Intent to Diagnose	416	319
Evaluable Subjects	383	298

5 undetermined reference test results (permanent section H&E/IHC) excluded.

Focus will be on ITD analyses.

Se, Sp were the primary endpoints.
PPV, NPV, ROC curve also considered.

Study Results, All Subjects

Intent to Diagnose (N=416)

Invalid test results treated as test –.

```
                    BLN Assay
                   -        +
       H&E/  –    278      17    295
       IHC   +     15     106    121
                  293     123    416
```

```
       Est. 95% CI
Se   87.6 80.4,92.9
```

Study Results, All Subjects

Intent to Diagnose (N=416)

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                    BLN Assay
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      H&E/  -    278      17    295
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```

```
         Est.  95% CI
Se    87.6  80.4,92.9
Sp    94.2  90.9,96.6
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Study Results, All Subjects

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Se   87.6 80.4,92.9
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PPV  86.2 78.8,91.7
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Study Results, All Subjects

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        Est.  95% CI
Se   87.6  80.4,92.9
Sp   94.2  90.9,96.6
PPV  86.2  78.8,91.7
NPV  94.9  91.7,97.1
```

Study Results, All Subjects

Intent to Diagnose (N=416)

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```
                    BLN Assay
                  -       +
        H&E/  -   278     17   295
        IHC   +    15    106   121
                  293    123   416
```

```
        Est.  95% CI      Bayes Decision Rule
Se   87.6 80.4,92.9  Pr(Se ≥ 0.7)=1.000
Sp   94.2 90.9,96.6  Pr(Sp ≥ 0.9)=0.999
PPV  86.2 78.8,91.7
NPV  94.9 91.7,97.1
```

Both Se, Sp hypotheses were met.

Study Results, All Subjects

Intent to Diagnose (N=416)

Invalid test results treated as test –.

```
                    BLN Assay
                    -       +
        H&E/  -   278     17    295
        IHC   +    15    106    121
                  293    123    416
```

	Est.	95% CI	Decision Rule
Se	87.6	80.4,92.9	$Pr(Se \geq 0.7)=1.000$
Sp	94.2	90.9,96.6	$Pr(Sp \geq 0.9)=0.999$
PPV	86.2	78.8,91.7	
NPV	94.9	91.7,97.1	Prevalence = 29.1

Both Se, Sp hypotheses were met.

Interpretation of ITD Results

Prevalence of ≥ 0.2 mm disease   29.1% (121/416)
Prevalence in test + subset (PPV) 86.2% (106/123).

```
                    BLN Assay
                  -        +
      H&E/  -    278      17    295
      IHC   +     15     106    121
                 293     123    416
```

Interpretation of ITD Results

Prevalence of ≥ 0.2 mm disease   29.1% (121/416)
Prevalence in test + subset (PPV) 86.2% (106/123).

13.8% (17/123) <u>of test + subjects</u> would undergo ALND that was unsubstantiated by subsequent permanent section H&E.

```
              BLN Assay
              -       +
H&E/  -     278     17    295
IHC   +      15    106    121
            293    123    416
```

Interpretation of ITD Results

Prevalence of ≥ 0.2 mm disease   29.1% (121/416)
Prevalence in test + subset (PPV) 86.2% (106/123).

4.1% (17/416) of all subjects would undergo ALND that was unsubstantiated by subsequent permanent section H&E.

```
                BLN Assay
                 -       +
   H&E/  -     278      17     295
   IHC   +      15     106     121
               293     123     416
```

Interpretation of ITD Results

Prevalence of ≥ 0.2 mm disease   29.1% (121/416)
Prevalence in test + subset (PPV) 86.2% (106/123).

Suppose all subjects with positive permanent section H&E receive ALND **(29.1%, 121/416)**.

```
                    BLN Assay
                  -         +
     H&E/  -     278       17      295
     IHC   +      15      106      121
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```

Interpretation of ITD Results

Prevalence of ≥ 0.2 mm disease   29.1% (121/416)
Prevalence in test + subset (PPV) 86.2% (106/123).

Suppose all subjects with positive permanent section H&E receive ALND **(29.1%, 121/416)**. Then the rate of ALND surgeries would ↑ **4.1% (17/416),** from 29.1% to 33.2% (95%CI 31.5-35.6%).

```
                  BLN Assay
                   -      +
        H&E/  -   278    17    295
        IHC   +    15   106    121
                  293   123    416
```

Interpretation of ITD Results

Prevalence of < 0.2 mm disease   70.9% (295/416)
Prevalence in test – subset (NPV)94.9% (278/293).

		BLN Assay		
		–	+	
H&E/	–	278	17	295
IHC	+	15	106	121
		293	123	416

Interpretation of ITD Results

Prevalence of < 0.2 mm disease: 70.9% (295/416)
Prevalence in test – subset: 94.9% (278/293).

5.1% (15/293) <u>of test – subjects</u> are not referred to needed surgery unless & until disease is detected in permanent sections.

```
                BLN Assay
                 -       +
     H&E/  -    278     17    295
     IHC   +     15    106    121
                293    123    416
```

Interpretation of ITD Results

Prevalence of < 0.2 mm disease: 70.9% (295/416)
Prevalence in test – subset: 94.9% (278/293).

3.6% (15/416) of all subjects are not referred to needed surgery unless & until disease is detected in permanent sections.

```
                    BLN Assay
                   -       +
        H&E/  -   278     17   295
        IHC   +    15    106   121
                  293    123   416
```

Se, Sp, by Histologic Category

```
Histologic +      N TP   FN Se    (95%CI)
P(MA)(≥2.0 mm) 94 92    2 97.9 (92.5 - 99.7)
P     (≥0.2 mm)  4  1    3 25.0 ( 0.6 - 80.6)
P(MI)(0.2-2.0) 23 13   10 56.5 (34.5 - 76.8)


Histologic -      N FP   TN Sp    (95%CI)
N(CL) (<0.2mm)  6  1    5 83.3 (35.9 - 99.6)
N(ITC)(<0.2mm) 14  4   10 71.4 (41.9 - 91.6)
No tumor seen 275 12 263 95.6 (92.5 - 97.7)
```

The study was powered to detect .7 Se and .9 Sp overall, but not powered within histological categories.

Categories "P(MA)" and "No tumor seen" predominate.

ROC Curve Analysis

Non-diseased cases

Diseased cases

Score
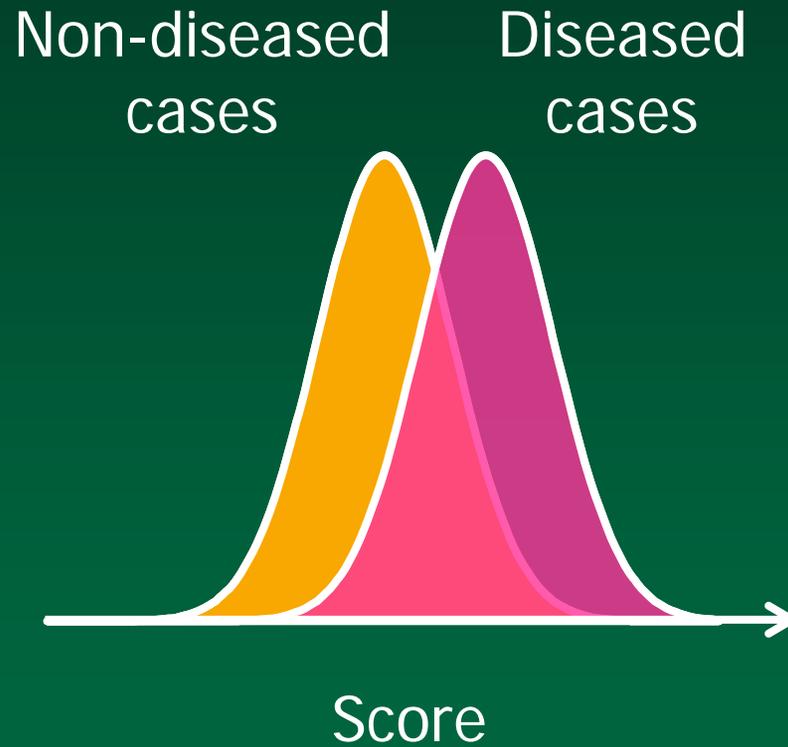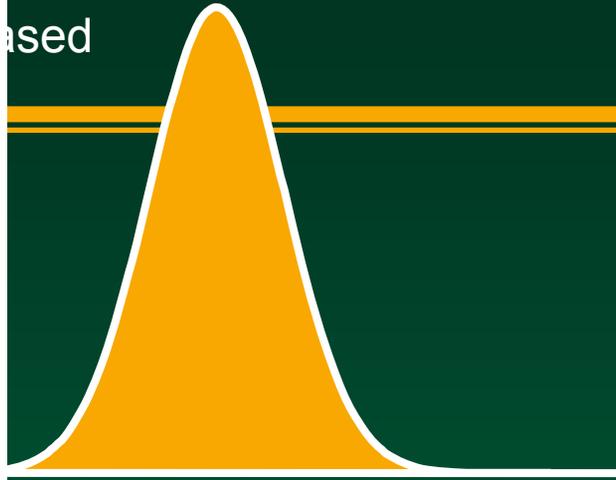
Non-diseased cases
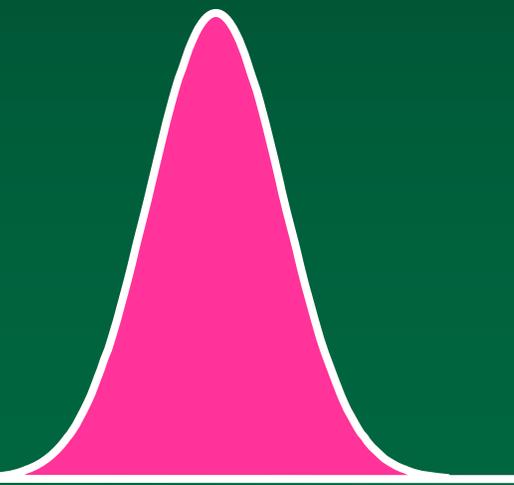
Single Threshold
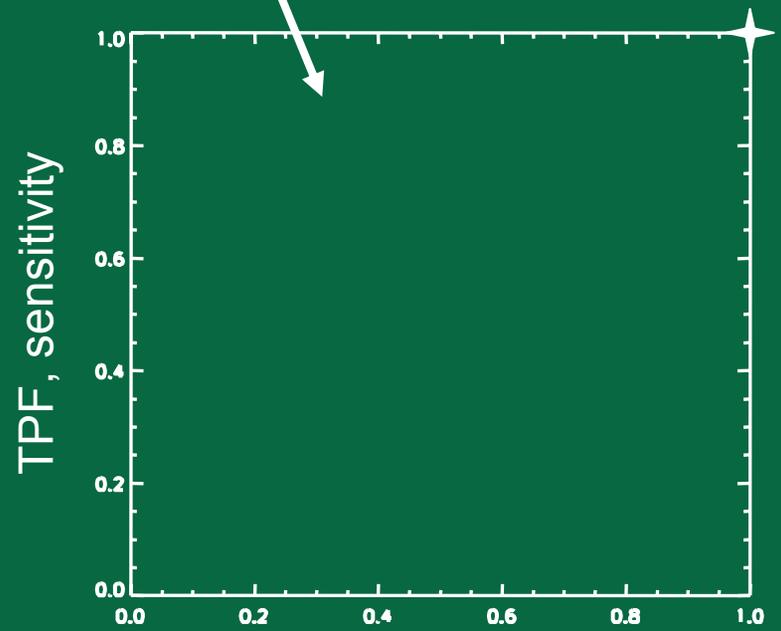
Diseased cases
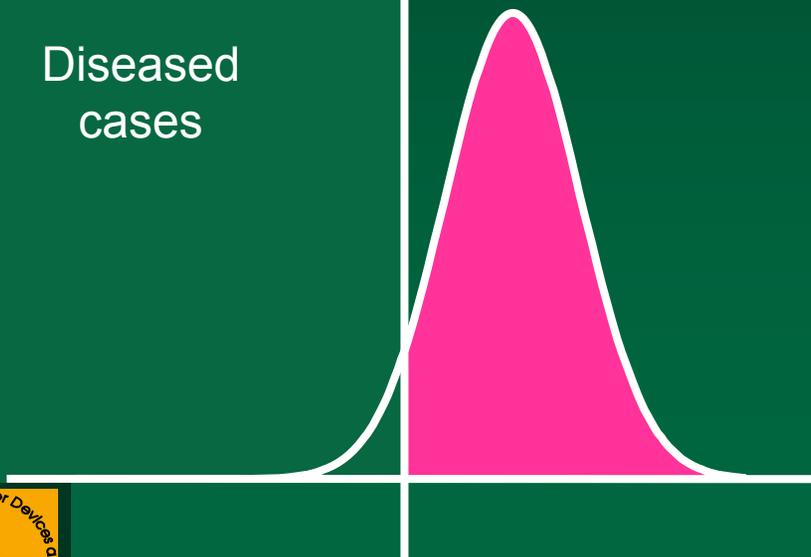
Single Operating Point

TP Rate, sensitivity

FP Rate, 1-specificity
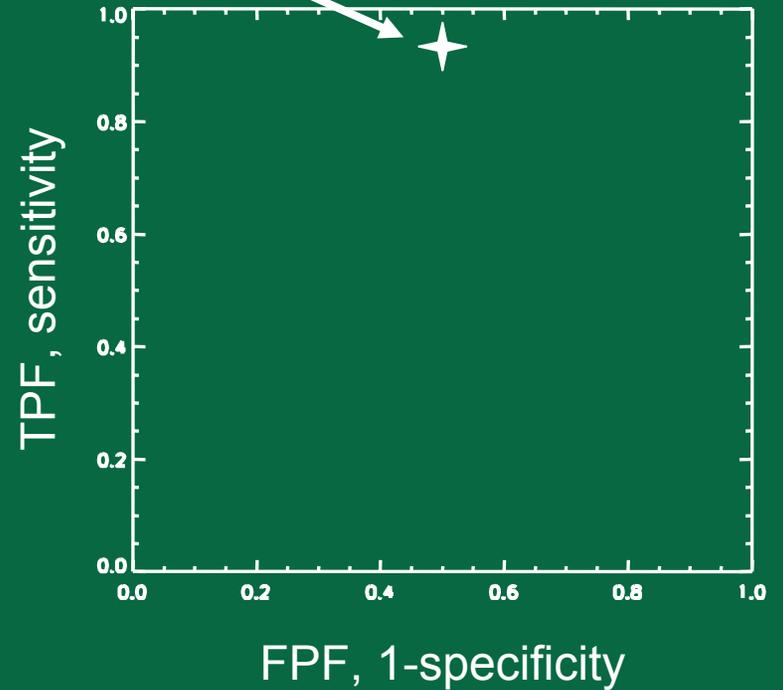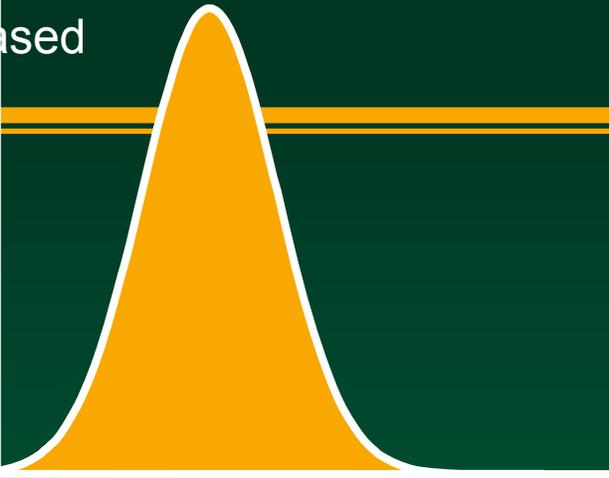
Non-diseased cases

Threshold Range

Diseased cases

Entire ROC Curve

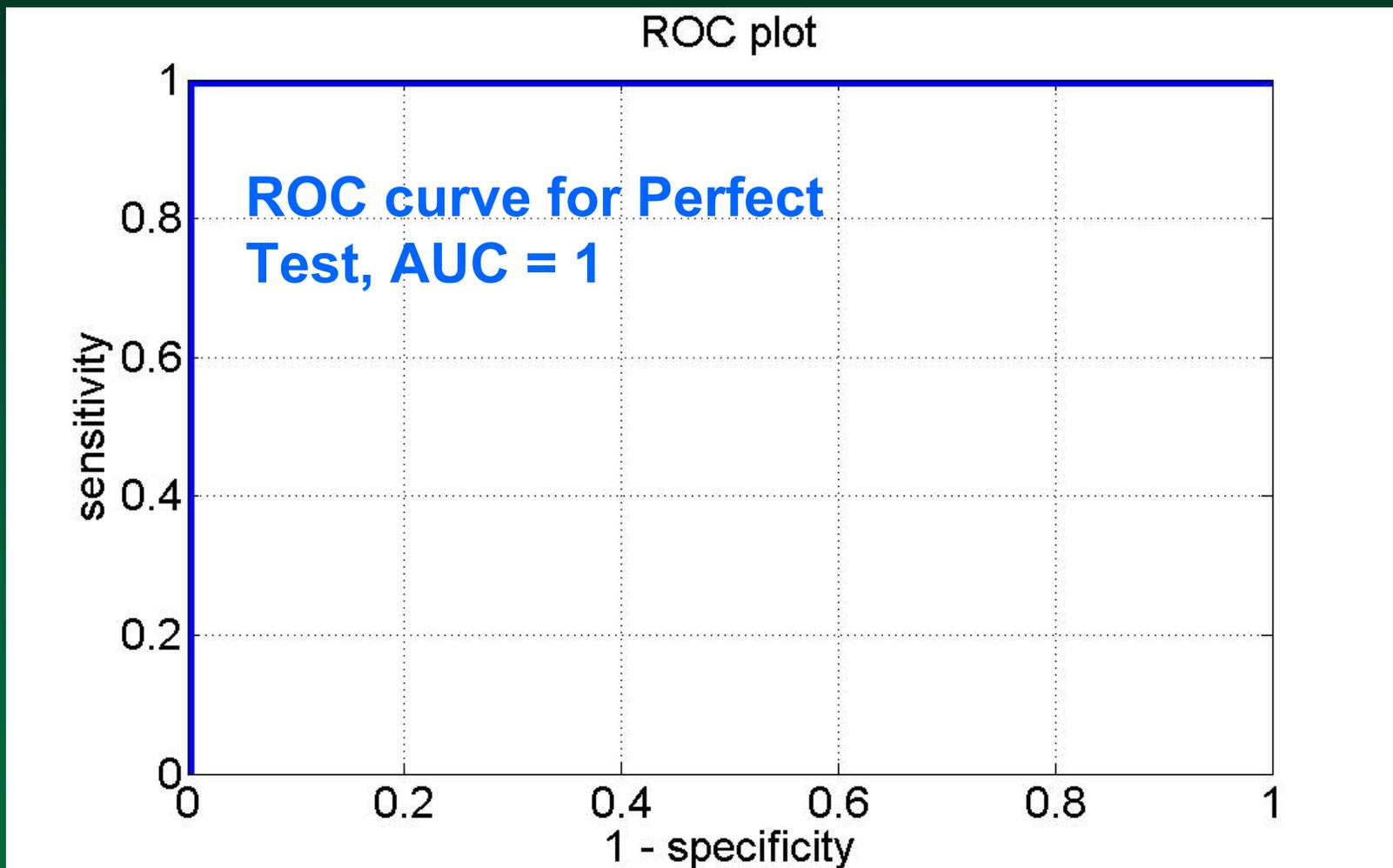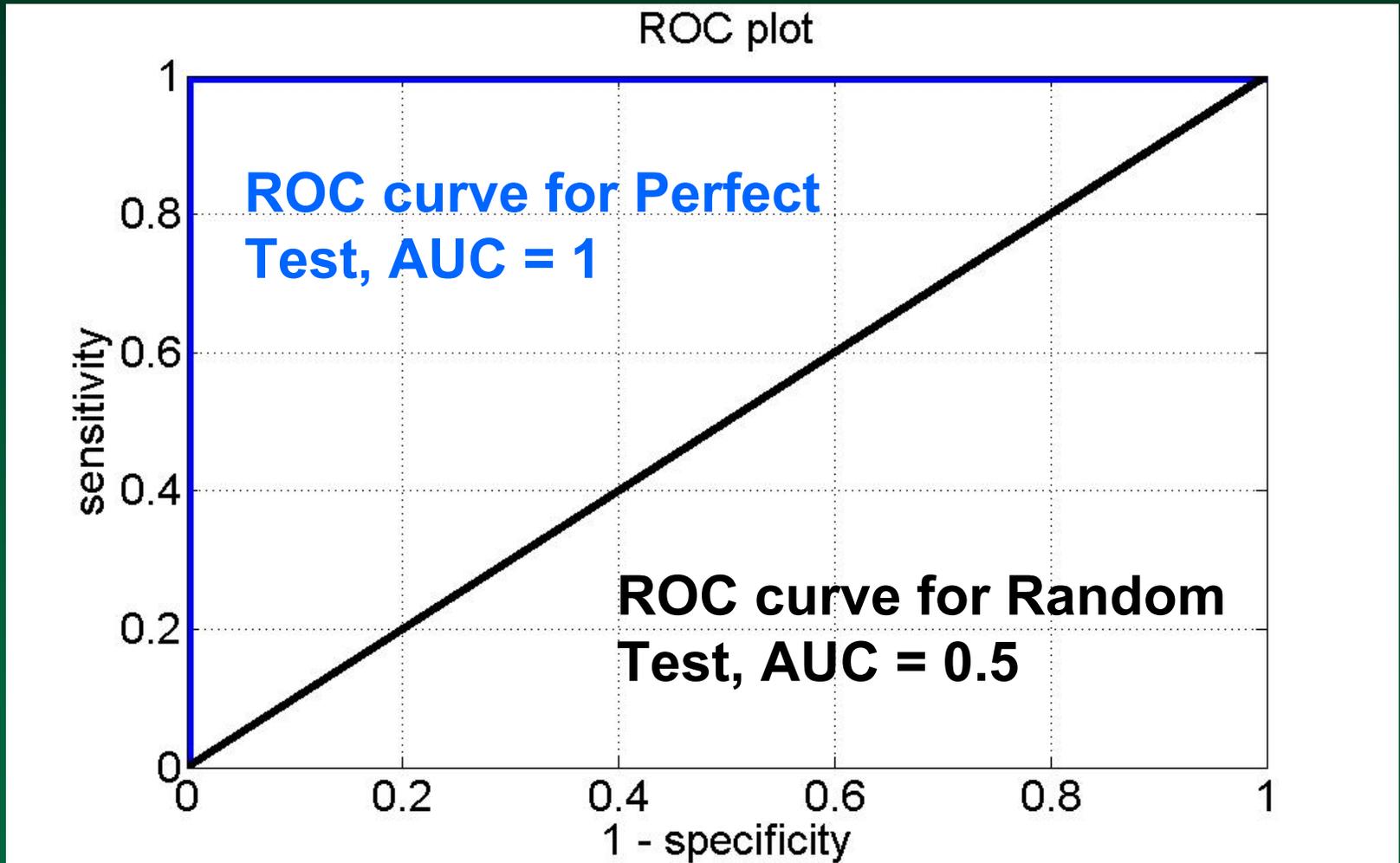TPF, sensitivity
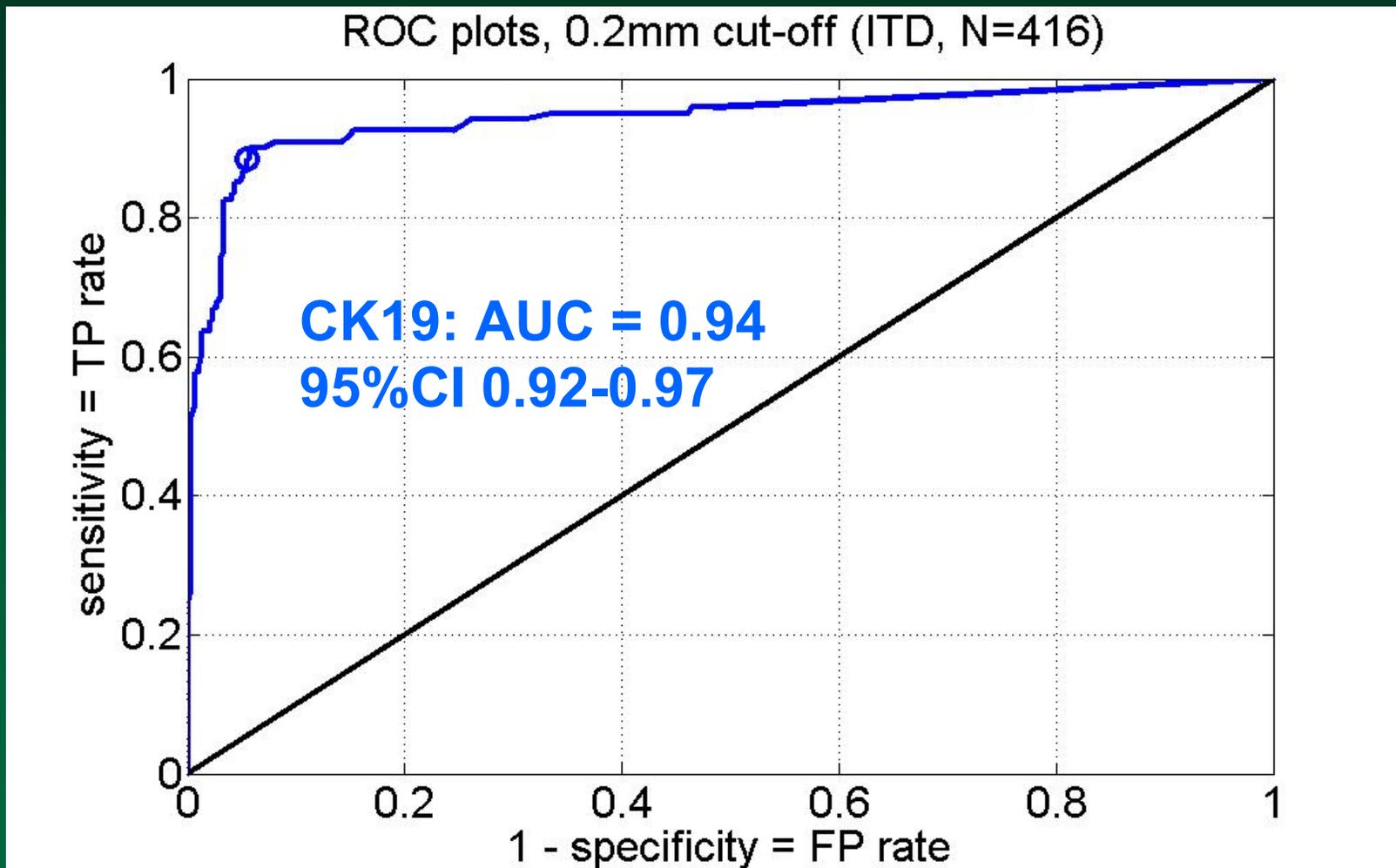
FPF, 1-specificity

ROC Curve, CK19 (N=416)

ROC Curve, CK19 (N=416)

ROC plot

ROC curve for Perfect Test, AUC = 1

ROC curve for Random Test, AUC = 0.5

sensitivity

1 - specificity

ROC Curve, CK19 (N=416)

ROC plots, 0.2mm cut-off (ITD, N=416)

CK19: AUC = 0.94
95%CI 0.92-0.97

ROC Curves, CK19 & MG (N=416)

ROC plots, 0.2mm cut-off (ITD, N=416)

CK19: AUC = 0.94
95%CI 0.92-0.97

MG: AUC = 0.88
95%CI 0.84-0.92
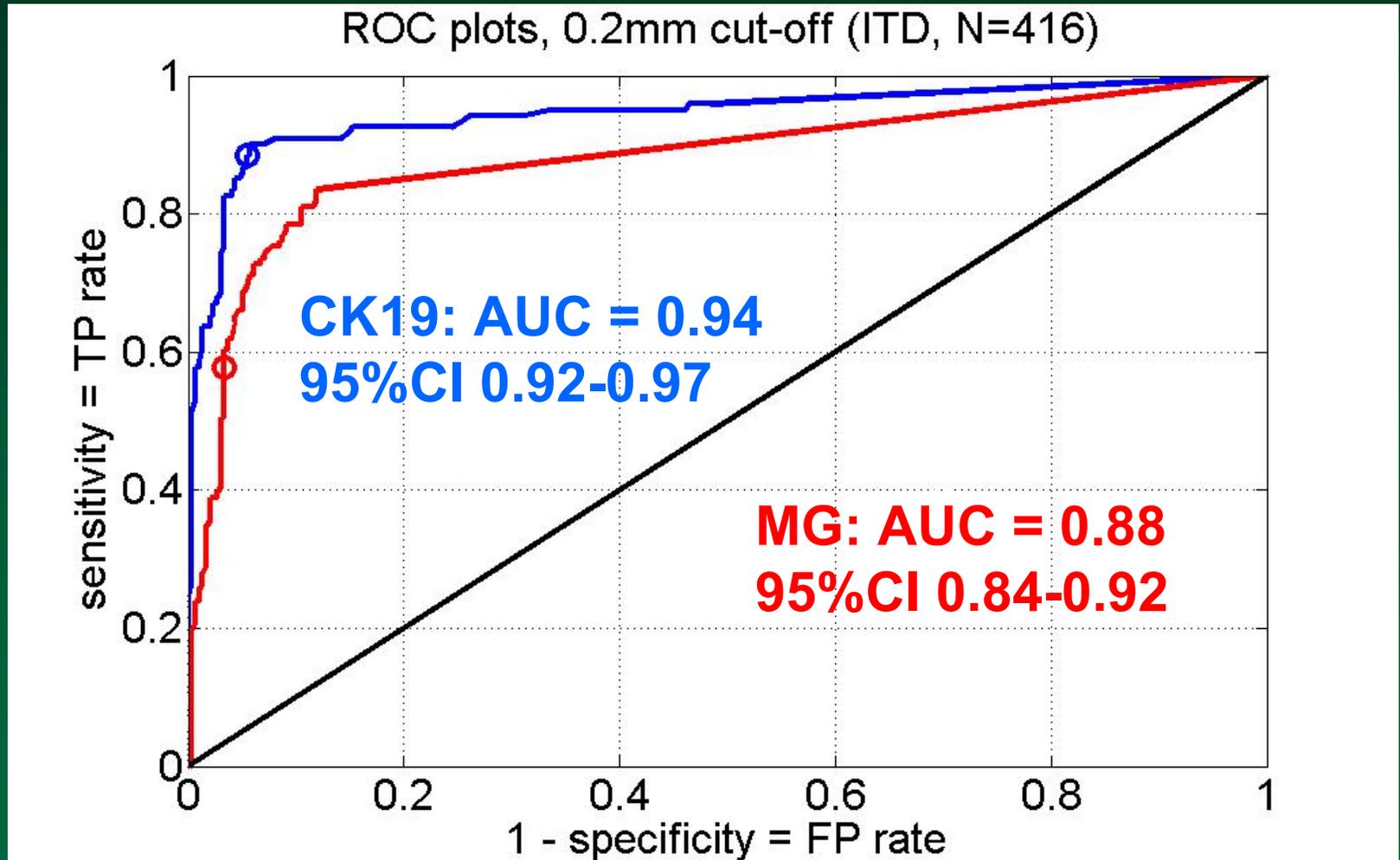
Study Results, FS H&E Subjects

FDA Analysis: BLN–FS Differences ITD (N=319)

	BLN	FS	Diff	FDA 95%CI	stat sign?
Se	95.6	85.6	10.0	2.5,17.7	yes
Sp	94.3	97.8	-3.5	-7.4, 0.0	brdln
PPV	86.9	93.9	-7.0	-14.8, 1.3	no
NPV	98.2	94.5	3.7	0.8, 6.4	yes

CK19 ROC plot vs. BLN vs FS (N=319)

ROC curve, CK19
AUC = .974

BLN Assay
Sp = 94.3
Se = 95.6

FS H&E
Sp = 97.8
Se = 85.6
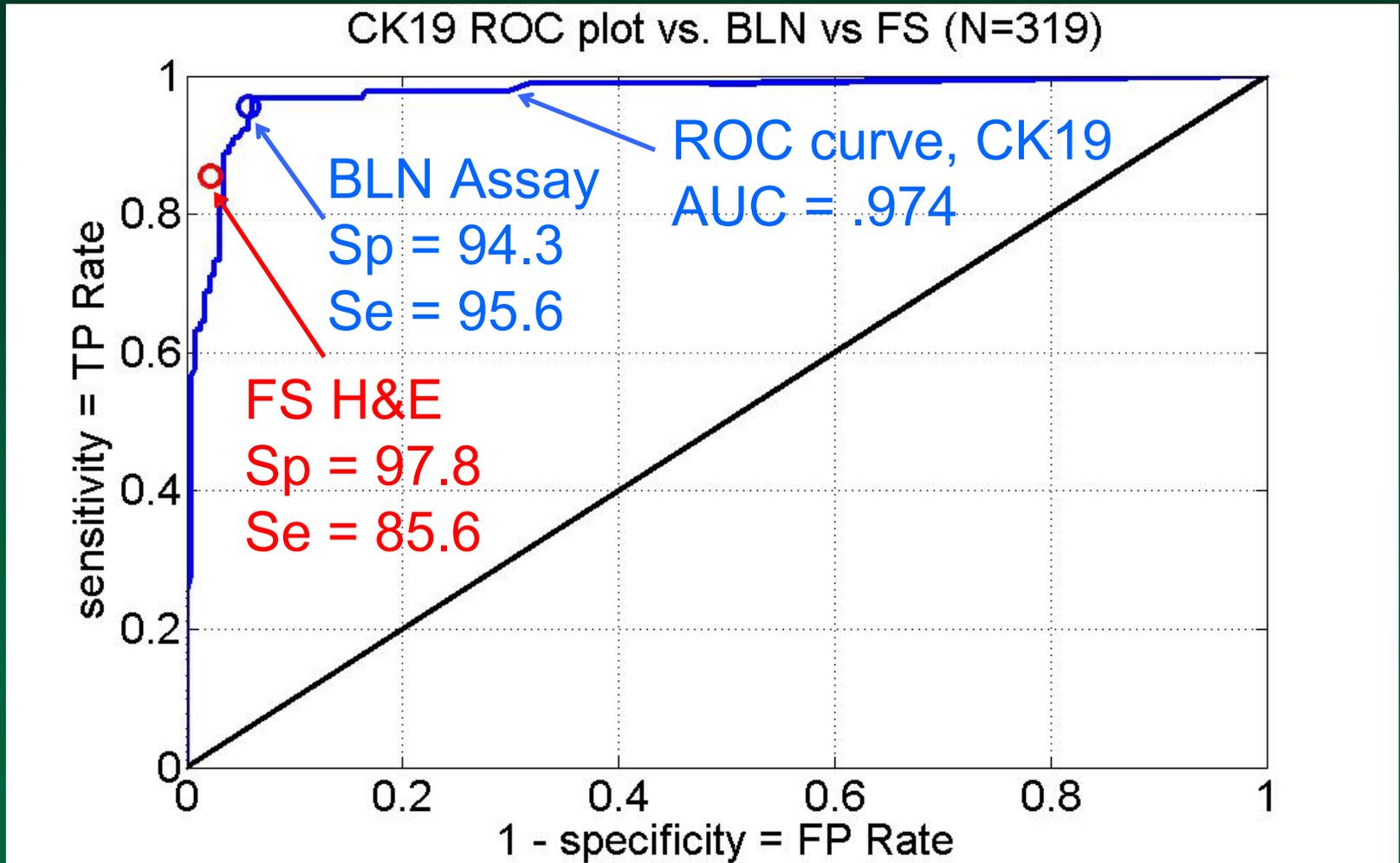
BLN Variability in Se, Sp by Site

Site	N	H&E/IHC +	H&E/IHC −	Se	Sp
1	28	12	16	100.0	100.0
2	28	12	16	91.7	93.8
3	11	5	6	60.0	83.3
4	63	18	45	77.8	97.8
6	23	10	13	70.0	84.6
7	124	33	90	100.0	92.2
9	11	4	7	50.0	100.0
10	7	1	6	100.0	100.0
11	10	3	7	66.7	100.0
13	34	6	27	83.3	92.6
14	82	17	62	94.1	95.2
	421	121	295	87.6	94.2

Breslow-Day p value for heterogeneity among sites (in odds ratio) is p=0.066.

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Summary

ITD analysis, all subjects: Hypotheses Se $\geq$ 0.70 & Sp $\geq$ 0.90 were both met.

BLN assay performance:
    Se 87.6 (80.4,92.9)   PPV 86.2 (78.8,91.7)
    Sp 94.2 (90.9,96.6)   NPV 94.9 (91.7,97.1)

Rate of ALND surgeries would increase from 29.1% to 33.2% (95%CI 31.5-35.6%).

Bulk of subjects were in 2 of 6 histological categories.

Summary

Comparison of BLN assay with FS H&E:

Se ↑ 10.0% (s)   PPV ↓ 7.0% (ns)
Sp ↓ 3.5% (bs)   NPV ↑ 3.7% (s)

FS and BLN appear to be operating at different points on the same (or similar) ROC curve.

Variation over sites: Heterogeneity in performance was borderline significant.