

Advanced Laboratory Medicine
Post-Analytic Issues:
Reference Ranges and Interpretation

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Reference Values

- A value or set of values used to interpret a laboratory result
- The reference values can be a single cut-off, a set of cut-offs, or a range of values containing 95% of the results from a reference population
- Reference values should be determined on a representative sample from the patient population on which the test will be used

Reference Values

- The most common definition of the Reference Range is the range of values containing the central 95% of the “healthy” population, i.e. the Reference Limits are the values at 2.5% and 97.5%
- This definition results in 5% of the “healthy” population being classified as “abnormal” or “positive”

“Normal Range”

- The term “Normal Range” should be avoided because it implies health and/or a normal distribution and neither might represent the significance of a test result or the distribution of test results

Types of Reference Ranges

- Sodium – Reference range determined on a reference population; physiologically determined so it should be independent of region and ethnicity
- TSH – Reference range determined by regional diet; transfer of reference range from iodine sufficient region might be appropriate
- Cholesterol – Reference ranges not relevant; level to treat determined by epidemiologic studies of risk

Reference Range Study

1. Select a reference group representative of the population that will be tested
2. The reference group should be free of disease and conditions that might cause an “abnormal” result
3. Establish criteria for excluding individuals with factors that may impact the test
4. Screen and test reference group
5. Calculate reference range

Analytical Methods

- N = 120 (allows 3 subjects/each 2.5%)
- First, eliminate Outliers
- Determine 2.5% and 97.5% reference values
 - Parametric: Calculate mean +/- 2 SD
 - Non-Parametric: rank order the results and find the 2.5% and 97.5% values

Frequency Distribution for Calcium Reference Range Study

Analyte	(mg/L)*	Women	Men	Combined
Calcium	88	1	0	1
	89	2 ^b	0	2
	90	1	0	1
	91	3	2	5 ^p
	92	11	1 ^b	12
	93	11	8	19
	94	8	6	14
	95	16	11	27
	96	16	12	28
	97	26	13	39
	98	8	16	24
	99	7	14	21
	100	3	7	10
	101	2	10	12
	102	3 ^c	11	14
	103	2	7 ^c	9 ^c
	104	0	1	1
	105	0	0	0
	106	0	1	1
Total		120	120	240

Non-Parametric Reference Value Cut-offs

- 2.5 %
 - rank value #3; N = 120
 - rank value #6; N = 240

- 97.5%
 - rank value #118; N = 120
 - rank value #235; N = 240

Frequency Distribution for Calcium Reference Range Study

Analyte	(mg/L)*	Women	Men	Combined
Calcium	88	1	0	1
	89	2 ^b	0	2
	90	1	0	1
	91	3	2	5 ^p
	92	11	1 ^b	12
	93	11	8	19
	94	8	6	14
	95	16	11	27
	96	16	12	28
	97	26	13	39
	98	8	16	24
	99	7	14	21
	100	3	7	10
	101	2	10	12
	102	3 ^c	11	14
	103	2	7 ^c	9 ^c
	104	0	1	1
	105	0	0	0
	106	0	1	1
	Total	120	120	240

Calcium Reference Limits and 90% Confidence Intervals

Non Parametric Reference Limits

– Women:	89	-	102 mg/L
– Men:	92	-	103 mg/L
– Combined:	91	-	103 mg/L

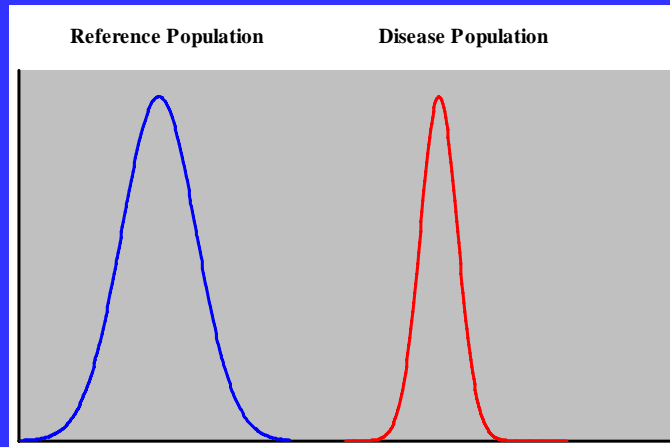
Confidence Interval for Reference Limits

– Women:	88 - 91	101 - 103 mg/L
– Men:	91 - 93	103 - 106 mg/L
– Combined:	88 - 91	103 - 106 mg/L

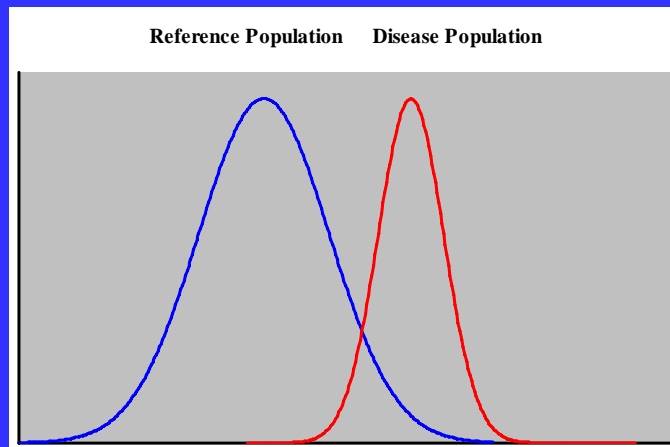
CUMC Coagulation Crossover Study

Nov-2004	PT CORE	PT ALLEN	PTT CORE	PTT ALLEN	FIBRINOGEN CORE	FIBRINOGEN ALLEN
Number	117	105	117	105	117	69
Min	12.8	12.3	26.0	25.3	208	225
Max	16.4	15.9	39.5	44.8	553	593
Median	13.9	13.4	30.4	30.9	337	374
Mean	14.0	13.5	30.7	31.6	339	370
Std Dev	0.7	0.7	2.9	3.7	61	72
Mean - 2SD	12.7	12.2	25.0	24.1	217	226
Mean + 2SD	15.4	14.8	36.5	39.0	461	514
2.50%	13.0	12.6	26.3	26.6	232	244
97.50%	16.0	15.3	37.5	40.2	447	512

Ideal World Reference and Disease Groups do not Overlap



Real World Overlapping Distributions



Types of Tests

- Screening
- Diagnostic
- Therapeutic Monitoring

Sensitivity and Specificity

Sensitivity: The probability that a patient who is disease positive will test positive

Specificity: The probability that a patient who is disease negative will test negative

Positive and Negative Predictive Values

Positive Predictive Value: The probability that a patient who is test positive is disease positive

Negative Predictive Value: The probability that a patient who is test negative is disease negative

Predictive Value Table

	Patients with disease	Patients without disease	
Test positive	TP	FP	→
Test negative	FN	TN	→
	↓	↓	

Predictive Value Table

	Prevalence	1-Prevalence	
	Patients with disease	Patients without disease	
Test positive	TP	FP	→ Positive Predictive Value
Test negative	FN	TN	→ Negative Predictive Value

↓ ↓
Sensitivity Specificity

Predictive Values as a Function of Sensitivity, Specificity, and Prevalence

$$PV_p = \frac{TP}{TP + FP}$$

$$PV_n = \frac{TN}{TN + FN}$$

Predictive Values as a Function of Sensitivity, Specificity, and Prevalence

$$PV_p = \frac{TP}{TP + FP} = \frac{p * Se}{p * Se + (1-p) * (1-Sp)}$$

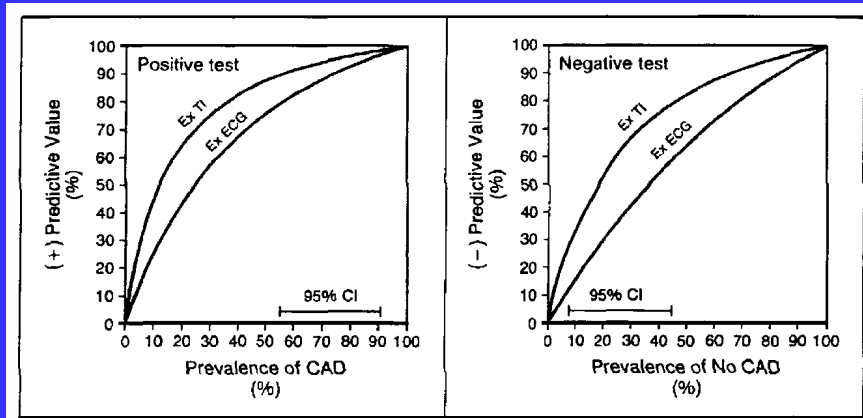
$$PV_n = \frac{TN}{TN + FN} = \frac{(1-p) * Sp}{(1-p) * Sp + p * (1-Se)}$$

Comparison of the Predictive Power of 3 Hypothetical Diagnostic Tests

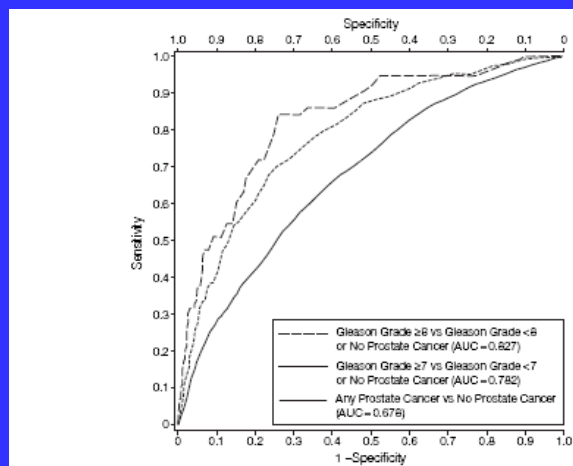
Test	Sensitivity	Specificity
A	0.95	0.81
B	0.85	0.83
C	0.75	0.85

It is impossible to compare the predictive power of these tests without knowing the prevalence and the predictive power will change as the prevalence changes.

Predictive Values as a Function of Prevalence



Receiver Operating Characteristic for PSA

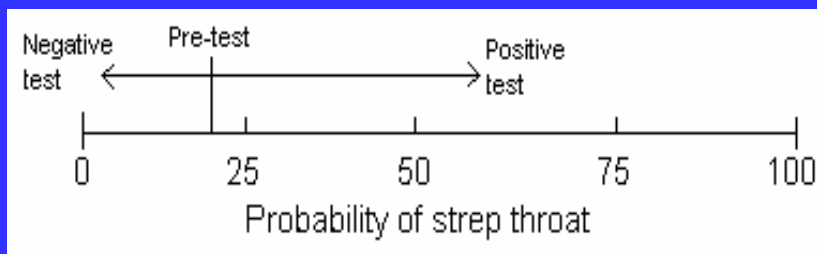


AUC indicates area under the receiver operating characteristic curve.

Likelihood Ratios

- We take our initial assessment of the likelihood of disease (“pre-test probability”), do a test to help us shift our suspicion one way or the other, and then determine a final assessment of the likelihood of disease (“post-test probability”).
- The likelihood ratio incorporates both the sensitivity and specificity of the test and provides a direct estimate of how much a test result will change the odds of having a disease.
- (+)LR, the likelihood ratio for a positive result, indicates how much the odds of the disease increase when a test is positive.
- (-)LR, the likelihood ratio for a negative result, indicates how much the odds of the disease decrease when a test is negative.

How LR(+) and LR(-) Shift the Probability of Disease



The “Positive Likelihood Ratio” (LR+) tells us how much to increase the probability of disease if the test is positive.

The “Negative Likelihood Ratio” (LR-) tells us how much to decrease it if the test is negative.

Definition of Likelihood Ratios

(+)LR =

probability of a patient **with** the disease having a + test
probability of a patient **without** the disease having a + test

$$= \text{Se}/(1-\text{Sp})$$

(-)LR =

probability of a patient **with** the disease having a - test
probability of a patient **without** the disease having a - test

$$= (1-\text{Se})/\text{Sp}$$

Relationship of Predictive Values to Likelihood Ratios

$$\text{PVP} = \frac{\text{TP}}{\text{TP} + \text{FP}} = \frac{p*\text{Se}}{p*\text{Se} + (1-p)*(1-\text{Sp})}$$

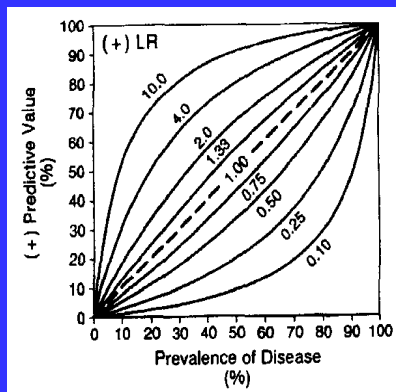
$$\text{PVN} = \frac{\text{TN}}{\text{TN} + \text{FN}} = \frac{(1-p)*\text{Sp}}{(1-p)*\text{Sp} + p*(1-\text{Se})}$$

Relationship of Predictive Values to Likelihood Ratios

$$\begin{aligned}
 \text{PVP} &= \frac{\text{TP}}{\text{TP} + \text{FP}} = \frac{p * \text{Se}}{p * \text{Se} + (1-p) * (1-\text{Sp})} \\
 &= \frac{p}{p + (1-p) * \frac{(1-\text{Sp})}{\text{Se}}}
 \end{aligned}$$

$$\begin{aligned}
 \text{PVN} &= \frac{\text{TN}}{\text{TN} + \text{FN}} = \frac{(1-p) * \text{Sp}}{(1-p) * \text{Sp} + p * (1-\text{Se})} \\
 &= \frac{(1-p)}{(1-p) + p * \frac{(1-\text{Se})}{\text{Sp}}}
 \end{aligned}$$

Predictive Value as a Function of Prevalence for Different Likelihood Ratios



Comparison of the Predictive Power of 3 Hypothetical Diagnostic Tests

Test	Sensitivity	Specificity	(+)LR	(-)LR
A	0.95	0.81	5.0	0.06
B	0.85	0.83	5.0	0.18
C	0.75	0.85	5.0	0.29

The Likelihood values clarify the relative rule-in (positive) and rule-out (negative) power of these tests for all levels of prevalence.

Interpretation of Likelihood Results

LR	Interpretation
> 10	Large and often conclusive increase in the likelihood of disease
5 - 10	Moderate increase in the likelihood of disease
2 - 5	Small increase in the likelihood of disease
1 - 2	Minimal increase in the likelihood of disease
1	No change in the likelihood of disease
0.5 - 1.0	Minimal decrease in the likelihood of disease
0.2 - 0.5	Small decrease in the likelihood of disease
0.1 - 0.2	Moderate decrease in the likelihood of disease
< 0.1	Large and often conclusive decrease in the likelihood of disease

Calculation of Post-Test Odds

- The likelihood ratio has an interesting property:

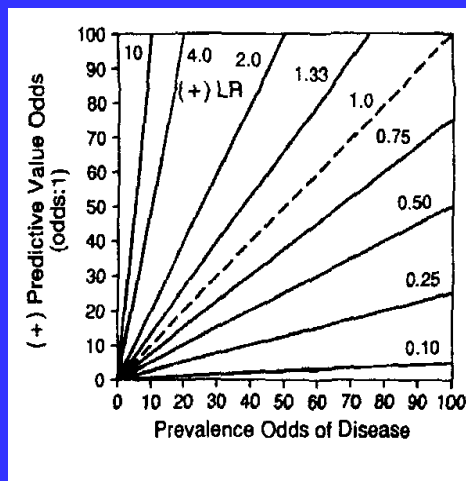
$$\text{Post-test odds of disease} = \text{likelihood ratio} * \text{pre-test odds of disease}$$

- So, for positive and negative tests:

$$\text{odds of disease for (+) test} = \text{odds of disease before testing} * (+)\text{LR}$$

$$\text{odds of disease for (-) test} = \text{odds of disease before testing} * (-)\text{LR}$$

Predictive Value versus Odds of Disease



Probability versus Odds

- The terms "odds of disease" and "probability of disease" are not the same thing.
- Consider a group of 10 patients, 3 have strep and 7 don't have strep
- The **probability** that a patient in this group has strep is $3/10$ or 0.3 or 30%.
- On the other hand, the **odds** of having strep in this group are 3 : 7

Converting Probability to Odds

Probability	Odds
1%	1:99
5%	1:19
10%	1:9
20%	1:4
33%	1:2
50%	1:1
67%	2:1
80%	4:1
90%	9:1
99%	99:1

- for an odds of a : b,
probability = $a / (a + b)$
- for a probability of x%, the
odds are x : (100 - x)

Calculation of Post-Test Odds

Step	Description
1.	Convert the pre-test probability to odds form
2.	Multiply the pre-test odds by the LR to calculate the post-test odds
3.	Convert the post-test odds back to a probability

Example Calculation

pre-test probability = 40%

(+)LR = 9

Step	Description	Calculation
1.	Convert the pre-test probability to odds form	$40\% = 40 / (100-40)$ $= 40 : 60 = 4 : 6$
2.	Multiply the pre-test odds by the LR to calculate the post-test odds	$(4 : 6) \times 9 = 36 : 6$
3.	Convert the post-test odds back to a probability	$36 : 6 = 36 / (36 + 6) = 36/42$ $= 0.86$ or 86%

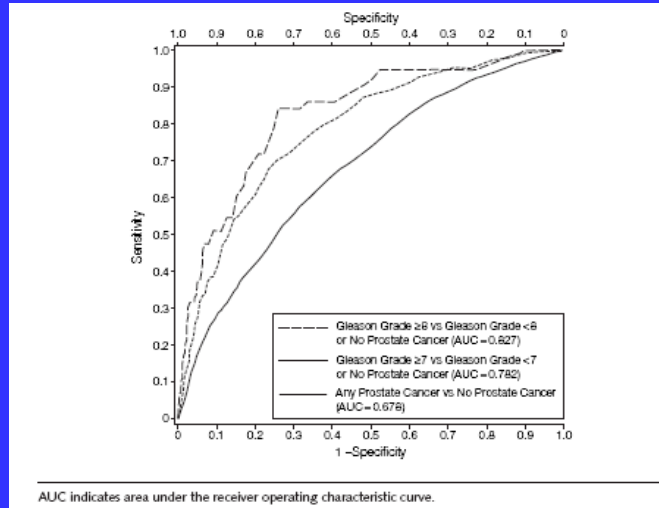
PSA Screening

Any Cancer (n = 1225) vs No Cancer (n = 4362)		
PSA	Sensitivity	Specificity
1.1	83.4	38.9
1.6	67.0	58.7
2.1	52.6	72.5
2.6	40.5	81.1
3.1	32.2	86.7
4.1	20.5	93.8
6.1	4.6	98.5
8.1	1.7	99.4
10.1	0.9	99.7

PSA Screening

Any Cancer (n = 1225) vs No Cancer (n = 4362)					p=.28		p=.20	
PSA	Sensitivity	Specificity	(+) LR	(-) LR	PVP	PVN	PVP	PVN
1.1	83.4	38.9	1.36	0.43	0.35	0.86	0.25	0.90
1.6	67.0	58.7	1.62	0.56	0.39	0.82	0.29	0.88
2.1	52.6	72.5	1.91	0.65	0.43	0.80	0.32	0.86
2.6	40.5	81.1	2.14	0.73	0.46	0.78	0.35	0.85
3.1	32.2	86.7	2.42	0.78	0.49	0.77	0.38	0.84
4.1	20.5	93.8	3.31	0.85	0.56	0.75	0.45	0.83
6.1	4.6	98.5	3.07	0.97	0.54	0.73	0.43	0.81
8.1	1.7	99.4	2.83	0.99	0.53	0.72	0.41	0.80
10.1	0.9	99.7	3.00	0.99	0.54	0.72	0.43	0.80

Receiver Operating Characteristic for PSA



Discussion