LABORATORY CALCULATIONS

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Learning Objectives

- Understand and be able to use the following types of calculations:
  - Reference intervals
  - Sensitivity/specificity
  - ROC curve
  - Student t test
  - Volume of distribution
  - Beer's Law
  - Enzyme kinetics
  - Basic management calculations
  - Buffers

Reference intervals

- Validating a reference interval?
- Transferring a reference interval?
- Establishing a reference interval
  - On a test with well-defined inclusion/exclusion criteria? - a priori sampling
  - On a new analyte? – a posteriori sampling

Reference intervals

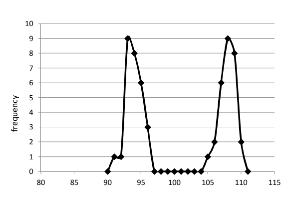
- Validating a reference interval?
  - 20 – 60 reference individuals
- Transferring a reference interval?
  - 20 – **60** reference individuals
- Establishing a reference interval
  - On a test with well-defined inclusion/exclusion criteria? - a priori sampling – 120 healthy individuals in each partition to get 90% C.I. at 95th percentile
  - On a new analyte? – a posteriori sampling – as many as you can analyze

Reference intervals

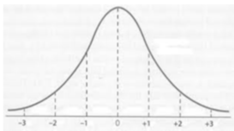
- Establishing a reference interval
- **Look at data distribution!** – why?
- Example: Chloride on CAVH fluid
  - N = 56
  - Mean = 101
  - Median 100
  - SD = 7

Reference intervals

- Chloride on CAVH fluid
  - N = 56
  - Mean = 101
  - Median 100
  - SD = 7



Reference intervals

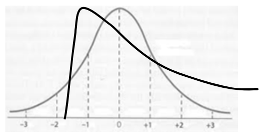


Normally distributed data:
use parametric statistics

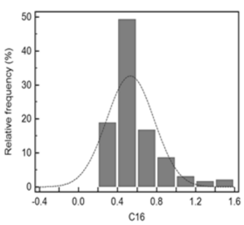
mean ± 2SD to get 95%

NOT normally distributed data:
use **non-parametric statistics**

2.5th and 97.5th percentiles

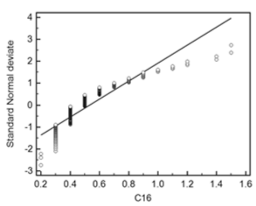


Reference interval for 3-OH-C16



Frequency histogram

Normal plot



Reference interval for 3-OH-C16

- Non-parametric analysis:
  - Rank the values in order, lowest to highest, and number them (1 = lowest value)
  - Determine 2.5th percentile and 97.5th percentile value
    - 2.5th = 0.025 (n+1)   97.5th = 0.975(n + 1)

3-OH-C16 reference interval

- N = 197
- Range = 0.2 – 1.5
- Mean = 0.53; median = 0.50

- Non-parametric 95% reference interval:
  - 2.5^{th} = 0.025(198) = 4.95 = 5^{th} value
  - 97.5^{th} = 0.975(198) = 193^{rd} value

0.3 – 1.2

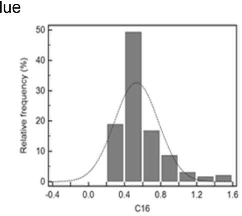
3-OH-C16 reference interval

- Non-parametric 95% reference interval:
  - 2.5^{th} = 0.025(198) = 4.95 = 5^{th} value
  - 97.5^{th} = 0.975(198) = 193^{rd} value

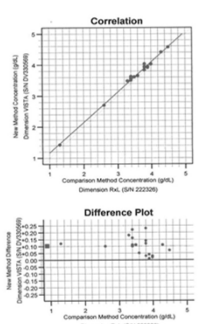
0.3 – 1.2

- Gaussian 95% interval

0.05 – 1.01



Validating a reference interval



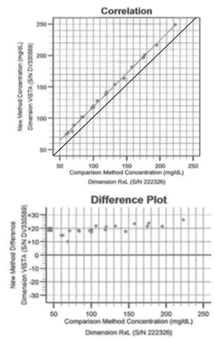
Y = 0.98 X + 0.19
r^2 = 0.9960

Slope almost 1.0, intercept almost 0

Average difference = +0.1 g/dL

Accepted current reference interval for use with new assay

Transferring a reference interval



Y = 1.063X + 9.1
$r^2 = 0.9998$

Average difference = +19 mg/dL

Adjusted reference intervals
Notified physicians

Clinical validity / utility of a test: sensitivity/specificity/predictive values

- **Specificity:** the frequency of a negative test when no disease is present; ability to rule out a disease

$$\textbf{Spec.} = \frac{TN}{TN + FP} \times 100 = (\%)$$

- **Sensitivity:** the frequency of a positive test when disease is present; ability of test to detect disease

$$\textbf{Sens.} = \frac{TP}{TP + FN} \times 100 = (\%)$$

Sensitivity/specificity

$$\text{Spec.} = \frac{TN}{TN + FP} \times 100 = (\%) \qquad \text{Sens.} = \frac{TP}{TP + FN} \times 100 = (\%)$$

3-OHFAs data – good test for diagnosing LCHAD and SCHAD?

SCHAD			LCHAD		
	SCHAD	No SCHAD		LCHAD	No LCHAD
Positive	6 (TP)	15 (FP)	Positive	8 (TP)	0 (FP)
Negative	0 (FN)	182 (TN)	Negative	0 (FN)	197 (TN)

Spec for SCHAD = 182/197 X100 = 92.4%

Sens for SCHAD = 6/6 X100 = 100%

Spec for LCHAD = 197/197 X100 = 100%

Sens for LCHAD = 8/8 X100 = 100%

Clinical/diagnostic utility

- **Positive predictive value (PPV)** – predictive value of a positive test

$$PPV = \frac{TP}{TP + FP} \times 100 = \quad \%$$

For SCHAD: 6/21 X 100 = 28.6%
For LCHAD: 8/8 X 100 = 100%

In general, if prevalence of disease is very low, get more FP, and PPV is bad

- **Negative predictive value (NPV) –** predictive value of a negative test

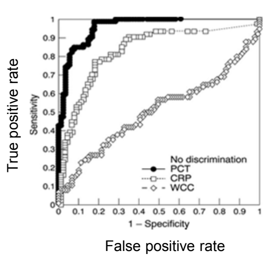
$$NPV = \frac{TN}{TN + FN} \times 100 = \quad \%$$

For SCHAD: 199/199 X100 = 100%
For LCHAD: 197/197 X100 = 100%
test good for ruling out both disorders

ROC curves

- Graphical way to present sensitivity and specificity data
- Software also gives you:
  - PPV, NPV
  - Likelihood ratios: +LR, -LR – likelihood a pos test will be seen in a patient with the disease compared to a patient without the disease
    - ↑ +LR – the better the test is for diagnosing disease
    - ↑ -LR – the better the test is at ruling out the disease
- Sensitivity and specificity can be considered reciprocals

ROC curves

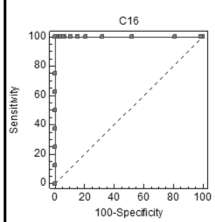


AUC = 1.00
perfect test
100% sensitive and specific

AUC = 0.500
test is no better than flipping a coin

To set up a ROC curve
- For each data point, assign a 1 (disorder present) or a 0, (disorder absent)

ROC curves - LCHAD



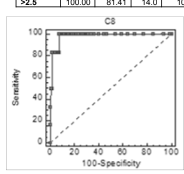
AUC = 1.000

Criterion	Sens	Spec	+PV	-PV	+LR	-LR
≥0.2	100.00	0.00	3.9		1.00	
>0.2	100.00	1.52	4.0	100.0	1.02	0.00
>0.3	100.00	18.78	4.8	100.0	1.23	0.00
>0.4	100.00	47.72	7.2	100.0	1.91	0.00
>0.5	100.00	68.02	11.3	100.0	3.13	0.00
>0.6	100.00	79.19	16.3	100.0	4.80	0.00
>0.7	100.00	84.77	21.1	100.0	6.57	0.00
>0.8	100.00	89.34	27.6	100.0	9.38	0.00
>0.9	100.00	93.40	38.1	100.0	15.15	0.00
>1	100.00	94.92	44.4	100.0	19.70	0.00
>1.1	100.00	96.45	53.3	100.0	28.14	0.00
>1.2	100.00	97.97	66.7	100.0	49.25	0.00
>1.4	100.00	98.98	80.0	100.0	98.50	0.00
>1.5	100.00	100.00	100.0	100.0		0.00
>2.7	75.00	100.00	100.0	99.0		0.25
>3.4	62.50	100.00	100.0	98.5		0.37
>6	50.00	100.00	100.0	98.0		0.50
>6.3	37.50	100.00	100.0	97.5		0.62
>7.2	25.00	100.00	100.0	97.0		0.75
>21.3	12.50	100.00	100.0	96.6		0.88
>25.9	0.00	100.00		96.1		1.00

ROC curve: SCHAD

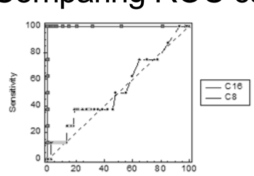
Criterion	Sens	Spec	+PV	-PV	+LR	-LR
≥0.2	100.00	0.00	2.9		1.00	
>0.2	100.00	1.51	3.0	100.0	1.02	0.00
>0.3	100.00	6.53	3.1	100.0	1.07	0.00
>0.4	100.00	15.08	3.4	100.0	1.18	0.00
>0.5	100.00	23.12	3.8	100.0	1.30	0.00
>0.6	100.00	30.15	4.1	100.0	1.43	0.00
>0.7	100.00	35.68	4.5	100.0	1.55	0.00
>0.8	100.00	41.21	4.9	100.0	1.70	0.00
>0.9	100.00	46.23	5.3	100.0	1.86	0.00
>1	100.00	53.27	6.1	100.0	2.14	0.00
>1.1	100.00	55.78	6.4	100.0	2.26	0.00
>1.2	100.00	59.80	7.0	100.0	2.49	0.00
>1.3	100.00	63.32	7.6	100.0	2.73	0.00
>1.4	100.00	66.33	8.2	100.0	2.97	0.00
>1.5	100.00	68.84	8.8	100.0	3.21	0.00
>1.6	100.00	71.36	9.5	100.0	3.49	0.00
>1.7	100.00	72.36	9.8	100.0	3.62	0.00
>1.8	100.00	72.86	10.0	100.0	3.69	0.00
>1.9	100.00	73.37	10.2	100.0	3.75	0.00
>2	100.00	74.37	10.5	100.0	3.90	0.00
>2.1	100.00	76.38	11.3	100.0	4.23	0.00
>2.2	100.00	76.88	11.5	100.0	4.33	0.00
>2.3	100.00	79.40	12.8	100.0	4.85	0.00
>2.4	100.00	80.40	13.3	100.0	5.10	0.00
>2.5	100.00	81.41	14.0	100.0	5.38	0.00

Criterion	Sens	Spec	+PV	-PV	+LR	-LR
>2.6	100.00	82.41	14.6	100.0	5.69	0.00
>2.9	100.00	83.92	15.8	100.0	6.22	0.00
>3	100.00	84.92	16.7	100.0	6.63	0.00
>3.2	100.00	85.43	17.1	100.0	6.86	0.00
>3.3	100.00	85.93	17.6	100.0	7.11	0.00
>3.4	100.00	86.93	18.8	100.0	7.65	0.00
>3.7	100.00	87.44	19.4	100.0	7.96	0.00
>3.8	100.00	88.44	20.7	100.0	8.65	0.00
>3.9	100.00	89.45	22.2	100.0	9.48	0.00
>4.1	100.00	90.45	24.0	100.0	10.47	0.00
>4.2	100.00	90.95	25.0	100.0	11.06	0.00
>4.3	100.00	91.96	27.3	100.0	12.44	0.00
>4.7	100.00	92.46	28.6	100.0	13.27	0.00
>4.8	83.33	92.46	25.0	99.5	11.06	0.18
>5.2	83.33	92.96	26.3	99.5	11.85	0.18
>5.3	83.33	93.47	27.8	99.5	12.76	0.18
>5.5	83.33	94.47	31.2	99.5	15.08	0.18
>5.8	83.33	94.97	33.3	99.5	16.58	0.18
>6.5	83.33	95.48	35.7	99.5	18.43	0.17
>6.6	83.33	95.98	38.5	99.5	20.73	0.17
>6.8	83.33	96.48	41.7	99.5	23.69	0.17
>7	83.33	97.49	50.0	99.5	33.17	0.17
>7.2	83.33	97.99	55.6	99.5	41.46	0.17
>8.2	83.33	98.49	62.5	99.5	55.28	0.17
>8.5	50.00	98.49	50.0	98.5	33.17	0.51
>8.8	50.00	98.99	60.0	98.5	49.75	0.51
>11.9	33.33	100.00	100.0	98.0		0.67
>29.4	16.67	100.00	100.0	97.5		0.83
>54.4	0.00	100.00		97.1		1.00

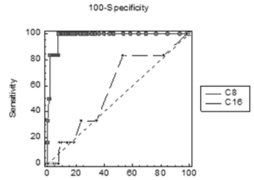


AUC = 0.982

Comparing ROC curves



For diagnosing LCHAD:
C16 AUC = 1.000
C8 AUC = 0.534



For diagnosing SCHAD:
C16 AUC = 0.581
C8 AUC = 0.982

Student t test

If comparing a sample with the population from which it was selected:

$$t = \frac{\overline{x} - \mu}{s/\sqrt{N}}$$

Or, if comparing two samples:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

Student t test

If comparing a sample with the population from which it was selected:

$$t = \frac{\overline{x} - \mu}{s/\sqrt{N}}$$

Average age of attendees at a conference is 32
The ages of the 10 attendees in the front row are 35, 37, 40, 30, 34, 35, 38, 32, 34 and 39. Are older attendees more likely to sit on the front row?

Mean = 35.4
s = 3.13
9 degrees of freedom

t = (35.4 – 32) ÷ (3.13/ √10)

= 3.4 ÷ (3.13/3.16)

= 3.4/0.99 = **3.4243**

Student t test

If comparing a sample with the population from which it was selected:

$$t = \frac{\overline{x} - \mu}{s/\sqrt{N}}$$

t = **3.4243**
9 degrees of freedom
(N – 1)

Older attendees are more likely to sit on the front row.

P = 0.0075

	1-tail: 0.25	0.1	0.05	0.025	0.01	0.005	0.001
d.f.	2-tail: 0.50	0.2	0.1	0.05	0.02	0.01	0.002
1	1.000	3.078	6.314	12.706	31.821	63.657	318.309
2	0.816	1.886	2.920	4.303	6.965	9.925	22.327
3	0.765	1.638	2.353	3.182	4.541	5.841	10.215
4	0.741	1.533	2.132	2.776	3.747	4.604	7.173
5	0.727	1.476	2.015	2.571	3.365	4.032	5.893
6	0.718	1.440	1.943	2.447	3.143	3.707	5.208
7	0.711	1.415	1.895	2.365	2.998	3.499	4.785
8	0.706	1.397	1.860	2.306	2.896	3.355	4.501
9	0.703	1.383	1.833	2.262	2.821	3.250	4.297
10	0.700	1.372	1.812	2.228	2.764	3.169	4.144
11	0.697	1.363	1.796	2.201	2.718	3.106	4.025
12	0.695	1.356	1.782	2.179	2.681	3.055	3.930
13	0.694	1.350	1.771	2.160	2.650	3.012	3.852
14	0.692	1.345	1.761	2.145	2.624	2.977	3.787
15	0.691	1.341	1.753	2.131	2.602	2.947	3.733
16	0.690	1.337	1.746	2.120	2.583	2.921	3.686
17	0.689	1.333	1.740	2.110	2.567	2.898	3.646
18	0.688	1.330	1.734	2.101	2.552	2.878	3.610
19	0.688	1.328	1.729	2.093	2.539	2.861	3.579
20	0.687	1.325	1.725	2.086	2.528	2.845	3.552
21	0.686	1.323	1.721	2.080	2.518	2.831	3.527
22	0.686	1.321	1.717	2.074	2.508	2.819	3.505
23	0.685	1.319	1.714	2.069	2.500	2.807	3.485
24	0.685	1.318	1.711	2.064	2.492	2.797	3.467
25	0.684	1.316	1.708	2.060	2.485	2.787	3.450
26	0.684	1.315	1.706	2.056	2.479	2.779	3.435
27	0.684	1.314	1.703	2.052	2.473	2.771	3.421
28	0.683	1.313	1.701	2.048	2.467	2.763	3.408
29	0.683	1.311	1.699	2.045	2.462	2.756	3.396
30	0.683	1.310	1.697	2.042	2.457	2.750	3.385

Student t test

Or, if comparing two samples:

$$t = \frac{X_1 - X_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

Measured 8 controls yesterday:
Mean = 8.7
S = 1.42

Measured 10 controls today:
Mean = 8.0
S = 0.79

Is there a significant bias between the two days?

Case 4: Student t test

Or, if comparing two samples:

$$t = \frac{X_1 - X_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

Measured 8 controls yesterday:
Mean = 8.7
S = 1.42

Measured 10 controls today:
Mean = 7.9
S = 0.86

$\sqrt{(1.42)^2/8 + (0.79)^2/10}$

$= \sqrt{0.252 + 0.062}$

$= 0.56$

$t = (8.7 – 8.0) \div 0.56$

$= 0.7 / 0.56$

= 1.25

Student t test

t = **1.25**
16 degrees of freedom
($N_1 + N_2 - 2$)

no significant bias between the 2 days

P = 0.1952

	1-tail: 0.25	0.1	0.05	0.025	0.01	0.005	0.001
d.f.	2-tail: 0.50	0.2	0.1	0.05	0.02	0.01	0.002
1	1.000	3.078	6.314	12.706	31.821	63.657	318.309
2	0.816	1.886	2.920	4.303	6.965	9.925	22.327
3	0.765	1.638	2.353	3.182	4.541	5.841	10.215
4	0.741	1.533	2.132	2.776	3.747	4.604	7.173
5	0.727	1.476	2.015	2.571	3.365	4.032	5.893
6	0.718	1.440	1.943	2.447	3.143	3.707	5.208
7	0.711	1.415	1.895	2.365	2.998	3.499	4.785
8	0.706	1.397	1.860	2.306	2.896	3.355	4.501
9	0.703	1.383	1.833	2.262	2.821	3.250	4.297
10	0.700	1.372	1.812	2.228	2.764	3.169	4.144
11	0.697	1.363	1.796	2.201	2.718	3.106	4.025
12	0.695	1.356	1.782	2.179	2.681	3.055	3.930
13	0.694	1.350	1.771	2.160	2.650	3.012	3.852
14	0.692	1.345	1.761	2.145	2.624	2.977	3.787
15	0.691	1.341	1.753	2.131	2.602	2.947	3.733
16	0.690	1.337	1.746	2.120	2.583	2.921	3.686
17	0.689	1.333	1.740	2.110	2.567	2.898	3.646
18	0.688	1.330	1.734	2.101	2.552	2.878	3.610
19	0.688	1.328	1.729	2.093	2.539	2.861	3.579
20	0.687	1.325	1.725	2.086	2.528	2.845	3.552
21	0.686	1.323	1.721	2.080	2.518	2.831	3.527
22	0.686	1.321	1.717	2.074	2.508	2.819	3.505
23	0.685	1.319	1.714	2.069	2.500	2.807	3.485
24	0.685	1.318	1.711	2.064	2.492	2.797	3.467
25	0.684	1.316	1.708	2.060	2.485	2.787	3.450
26	0.684	1.315	1.706	2.056	2.479	2.779	3.435
27	0.684	1.314	1.703	2.052	2.473	2.771	3.421
28	0.683	1.313	1.701	2.048	2.467	2.763	3.408
29	0.683	1.311	1.699	2.045	2.462	2.756	3.396
30	0.683	1.310	1.697	2.042	2.457	2.750	3.385

Volume of Distribution (V_d)

- The Volume of Distribution (V_d) is the amount of blood, per Kg body weight, necessary to contain all of the body burden of drug at equilibrium concentration.

$$Plasma\ Concentration = \frac{Total\ Body\ Stores}{Volume\ of\ Distribution}$$

Interpreting V_d

- Drugs with low V_d are contained mostly in the plasma, because . . .
  - They are highly water soluble (plasma water content is higher than tissues), or
  - They are highly protein bound (which prevents them from freely diffusing into tissues
- Drugs with high V_d are mostly in tissues, and plasma levels may not reflect body burden

V_d calculation

A 175 lb man takes a 5 mg dose of phenobarbital (V_d = 1.0 L/Kg). What is the *maximum* plasma phenobarbital concentration you can expect?

Plasma concentration = total body stores ÷ volume of distribution

175 lb = 79.4 Kg

C = (5 mg/79.4 Kg) ÷ 1.0 L/Kg

= 0.063 mg/Kg ÷ 1.0 L/Kg

= 0.063 mg/L = 0.063 μg/mL

V_d calculation

A 55 Kg woman has a plasma theophylline (V_d = 0.5 L/Kg) concentration of 15 µg/L. What is her total body burden of theophylline?

Plasma concentration = total body stores ÷ volume of distribution

15 µg/L = (concentration/55 Kg) ÷ 0.5 L/Kg

(15 µg/L)(0.5 Kg/L) = concentration/55 Kg

7.5 µg/Kg = concentration/55 Kg

(7.5 µg/Kg)(55 Kg) = concentration

412.5 µg

Beer's Law

- The mathematical formula that expresses:

concentration of an analyte dissolved in solution is directly proportional to it's absorbance.

Caveats:

1) Absorbance must be in the linear range (~0.05 - 2.0)
2) incident light must be monochromatic one wavelength
3) no interfering substances may be present absorbances are additive

Beer's law

A = abc

A = absorbance
a = absorptivity coefficient (ε = molar units)
b = path length of light through sample
c = concentration

Beer's Law

β-OH-butyrate + NAD^+ $\xleftrightarrow{\text{3-HBD}}$ acetoacetate + NADH + H^+

- 0.1 mL sample added to 2.7 mL buffer, 0.15 mL NAD^+ (27 mmol/L), and 50 µL 3-HBD
  (3 mL total volume: 0.1 + 2.7 + 0.15 + 0.05)
- Measured absorbance of produced NADH relative to a blank at 340 nm in a 1 cm cell
  A = 0.57
- Calculate the β-OH-butyrate concentration

β-OH-butyrate

- A = abc

A = absorbance = 0.57
ε = molar absorptivity of NADH
= 6.22×10^3 $L.mol^{-1}.cm^{-1}$
b = path length of light through sample = 1 cm
c = concentration

$0.57 = 6.22 \times 10^3 \times 1 \times c$

β-OH-butyrate

$0.57 = 6.22 \times 10^3 \times 1 \times c$

$c = 0.57 \div (6.22 \times 10^3) = 9.2 \times 10^{-5}$ mol/L

Convert to mmol/L (multiply x 10^3) = *0.092 mmol/L*

= β-OH-butyrate in final mixture!

Calculate β-OHB in sample by multiplying by dilution factor (V_T/V_s)

β-OH-butyrate

Total volume = 2.7 + 0.1 + 0.15 + 0.05 = 3.0 mL

(0.092 mmol/L x 3.0 mL) ÷ 0.1 mL

= *2.76* mmol/L β-OH-butyrate in the sample

Can do this in a single calculation

β-OH-butyrate

Can do this in a single calculation

$$c = \frac{(0.57)(10^3)(3.0)}{(6.22 \times 10^3)(0.1)}$$

Careful to include all dilution factors and unit conversion factors

Enzymes

- Beer's Law $A = abc$
  - also used for calculating enzyme concentrations

$(\Delta Abs/\varepsilon \times d)(10^6)(V_T/V_S) = U/L$

- where: ΔAbs = change in absorbance per minute
  ε = molar absorptivity of product
  d = path length of light through sample
  V_T/V_S = total volume/sample volume
  10^6 = conversion from mol/L to µmol/L
  U/L = µmol/min/L

Lactate Dehydrogenase

Lactate + NAD^+ $\xleftrightarrow{LD}$ pyruvate + NADH + H^+

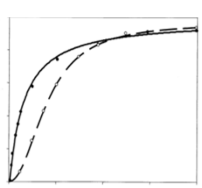
- 50 µL sample added to 1 mL of reagent containing buffer, NAD^+ and lactate
- Measure absorbance initially and at 1 minute intervals for 5 minutes in a cuvette with a 1 cm path length
- Calculate the LD enzyme activity (concentration)

LD

time	Abs
0	0.081
1	0.114
2	0.146
3	0.177
4	0.211
5	0.243

LD

time	Abs	ΔAbs
0	0.081	
1	0.114	0.033
2	0.146	0.032
3	0.177	0.031
4	0.211	0.034
5	0.243	0.032



0.162/5 = **0.032** = ΔAbs

LD

- $(\Delta Abs/\varepsilon \times d)(10^6)(V_T/V_S)$ = U/L

$$c = \frac{(0.032)(10^6)(1.05)}{(6.22 \times 10^3)(0.05)} = 108 \text{ U/L}$$

Enzyme Kinetics

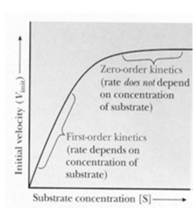
- **Kinetics** = mathematical description of a reaction as it is happening
- Michaelis and Menten developed a simple model for examining the kinetics of enzyme catalyzed reactions
  - ASSUMING:

    $E + S \leftrightarrow ES \rightarrow E + P$

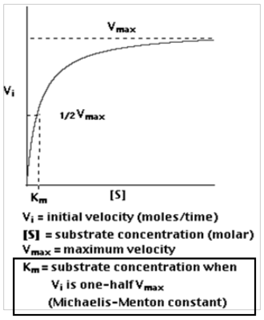
    - formation of ES is reversible
    - formation of E + P is irreversible
  - Michaelis-Menten plot

Enzyme Kinetics

- Velocity (rate) of the reaction:
  - **at low [S]:**
    ~straight line;
    1st order with respect to [S]
    -**velocity depends on [S]**
  - **at high [S]:**
    flat line
    zero order with respect to [S]
    Rate won't ⇑ no matter [S]
    unless enzyme concentration ↑
    -velocity depends on enzyme concentration



Michaelis-Menten Plot



$$V = \frac{V_{max}[S]}{K_m + [S]}$$

mathematical formula to describe this simple enzyme reaction

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

What is V_{max} ?

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

What is V_{max} ?

60 μmol/min

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

What is V_{max} ?

60 μmol/min

What is K_m ?

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

What is V_{max} ?

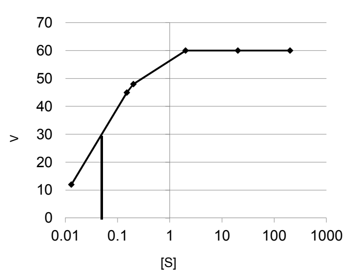
60 μmol/min

What is K_m ?

[S] at ½ V_{max}

[S] at 30 μmol/min

Michaelis-Menten plot



Draw M-M plot and determine K_m

Or calculate it.

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

$$45 = \frac{(60)(0.15)}{K_m + 0.15}$$

Enzyme Kinetics

V (μmol/min)	[S] (mmol/L)
60	200
60	20
60	2
48	0.2
45	0.15
12	0.013

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

$$45 = \frac{(60)(0.15)}{K_m + 0.15}$$

$$45\,(K_m + 0.15) = (60)(0.15)$$

$$45K_m + 45(0.15) = (60)(0.15)$$

$$45\,K_m + 6.75 = 9$$

$$45\,K_m = 9 - 6.75$$

$$45\,K_m = 2.25$$

$$K_m = \frac{2.25}{45}$$ = **0.05 mmol/L**

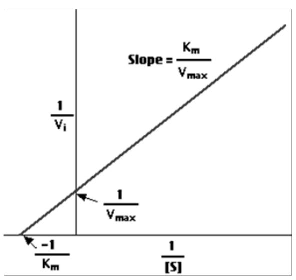
Lineweaver-Burk plot

- Since plot of V vs. [S] is not a straight line, it is difficult to obtain accurate values of Vmax and Km
- The Lineweaver-Burk plot or double reciprocal plot is a linear transformation of the Michaelis-Menten equation

$$\frac{1}{v} = \left\{\left(\frac{1}{[S]}\right)\left(\frac{Km}{Vmax}\right)\right\} + \left(\frac{1}{Vmax}\right)$$

This equation yields a straight line
Where: slope = Km/Vmax, y intercept = 1/Vmax, x intercept = -1/Km

Lineweaver-Burk plot

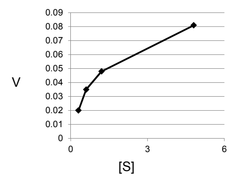


Lineweaver-Burk

[S]	V (ΔA)
0.3 mM	0.020
0.6 mM	0.035
1.2 mM	0.048
4.8 mM	0.081

Lineweaver-Burk

[S]	V (ΔA)
0.3 mM	0.020
0.6 mM	0.035
1.2 mM	0.048
4.8 mM	0.081



M-M plot

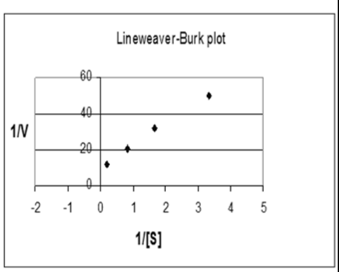
Lineweaver-Burk

[S]	V (ΔA)	1/[S]	1/V
0.3 mM	0.020	3.33	50
0.6 mM	0.035	1.67	31.7
1.2 mM	0.048	0.83	20.8
4.8 mM	0.081	0.21	12.3

plot

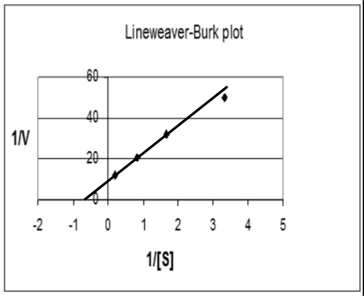
Lineweaver-Burk

1/[S]	1/V
3.33	50
1.67	31.7
0.83	20.8
0.21 1	2.3



Lineweaver-Burk experiment

1/[S]	1/V
3.33	50
1.67	31.7
0.83	20.8
0.21	12.3



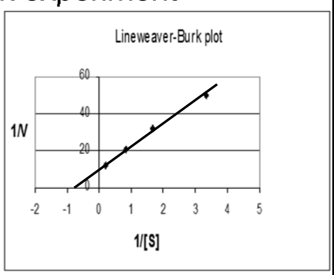
Lineweaver-Burk experiment

-1/Km = -0.7
-1/-0.7 = 1.43

Km = 1.43 mM

1/Vmax = 10
1/10 = 0.1

Vmax = 0.1 (abs/min)



Management – service contract?

- 75 i-stats at $9000.00 each
- Service contract: $30,000/year/20 I-stats
  - $70,000 to cover all 75 (instead of $82,500)
- Replacement cost of $2500.00/unit
- You have clumsy nurses and average needing to replace 16 units per year
- Do you need the service contract?
- What's the break even number of i-stats?

Management – service contract

- 75 i-stats at $9000.00 each
- Service contract: $30,000/year/ 20 I-stats to cover them, $70,000 to cover all 75.
- Replacement cost of $2500.00/unit
- You have clumsy nurses and average needing to replace 16 units per year

16 X 2500 = $40,000.00 Don't need a service contract.

70,000 ÷ 2500 = 28 Unless you start breaking more than 28 iStat/year, don't need a service contract

What if you had to buy a new i-Stat whenever you broke one? $9000 rather than $2500 per broken i-Stat

Management – service contract

- 75 i-stats at $9000.00 each
- Service contract: $30,000/year/ 20 I-stats to cover them, $70,000 to cover all 75.
- Replacement cost of $9,000/unit

16 X 9000 = $144,000.00 Yes! Need a service contract.

70,000 ÷ 9000 = 7.8 Unless you break less than 8 i-Stat/year, need a service contract

Management – bring that test in-house?

- Considerations –
  - Current cost to send test out
  - Current test volume
  - Current TAT and perceived needs
  - Tech time and workflow
  - Instrumentation to run assay
    - Newly available, FDA-approved assay on current chemistry platform
    - LDT assay on esoteric instrument

Management – bring that test in-house?

- Considerations –
  - Current cost to send test out
  - Current test volume
  - Current TAT and perceived needs
  - Tech time and workflow
  - Instrumentation to run assay
    - **Newly available assay on current chemistry platform**
    - Yes, unless:
      - Volume is so low, won't break even on what the test costs
      - Costs more on chem platform than sending it out
      - Volume is so high will impact workflow

Management – bring that test in-house?

- Voriconazole –
  - Current cost to send test out – $150.00/test
  - Current test volume – 1000/year
  - Current TAT and perceived needs – 4 days at best; want at least next day if possible
  - Tech time and workflow – limited techs on esoteric equipment
  - Instrumentation to run assay
    - **LDT assay on esoteric instrument – MS/MS assay**

MET - Voriconazole, plasma Waters Quattro

CDM Number	HCHG
CPT Code	80299
Date of Cost Study	6/23/2014
Cost Center Name	Metabolics and Advanced Diagnosti
Cost Center Number	53052
Methodology	MS/MS

Order Set Memberships

Order Set Add Total Cost

Costing tool

Testing Information

Number of Days/Week that Test is Run	3
Average Number of Runs per Week	3
Controls: Number of Days/Month	13
Calibration Curves: Average Number/Month	13
Number of Controls/Day	2
Average Number of Tests Billed/Month	15
Average Number of Dilutions/Month	0
Maximum Number of BT Tests/Run	16
Average Number of Repeats/Month	1
Number of PT and Parallel Tests/Month	1
Total BT/Year	2227

Testing and Hands On Tim

	Job Class or Grade	Minutes	Rate/Minute
Bench Tech Time/Calibration	C10	8	$0.500
Bench Tech Average Time/BT	C10	2	$0.500
Verification Time/BT	C10	2	$0.500
Start Up and Shut Down Time/BT	C10	24	$0.500
Supervisor Average Time/BT		0	$0.377
Lab Assistant Time/BT		0	$0.248
Nurse Time/BT		0	$0.548

Depreciation Cost

Instrument Name	Cost	Years	Cost/Yr
	$325,002.72	10	$32,500.27

Cost/Year	$32,500.27
Total BT/Year	2,227
Cost/BT	$14.59

Service Contract Cost

Contract Name	Cost	Years	Cost/Yr
	$63,105.42	1	$63,105.42

Cost/Year	$63,105.42
Total BT/Year	2,227
Cost/BT	$28.34

Lab Costs Database

Put in test information:

- Runs/week
- Calibrations/month
- Controls/month

- **Total billable tests (BT) per year**

Testing Information

Number of Days/Week that Test is Run	3
Average Number of Runs per Week	3
Controls: Number of Days/Month	13
Calibration Curves: Average Number/Month	13
Number of Controls/Day	2
Average Number of Tests Billed/Month	15
Average Number of Dilutions/Month	0
Maximum Number of BT Tests/Run	16
Average Number of Repeats/Month	1
Number of PT and Parallel Tests/Month	1
Total BT/Year	2227

Put in tech time

- Tech grade
- Time at various steps
- Anyone else's time

Testing and Hands On Tim

	Job Class or Grade	Minutes	Rate/M inute
Bench Tech Time/Calibration	C10	8	$0.500
Bench Tech Average Time/BT	C10	2	$0.500
Verification Time/BT	C10	2	$0.500
Start Up and Shut Down Time/BT	C10	24	$0.500
Supervisor Average Time/BT		0	$0.377
Lab Assistant Time/BT		0	$0.248
Nurse Time/BT		0	$0.548

Put in instrumentation costs

- Depreciation
- Service contract

Depreciation Cost

Instrument Name	Cost	Years	Cost/Yr
	$325,002.72	10	$32,500.27

Cost/Year	$32,500.27
Total BT/Year	2,227
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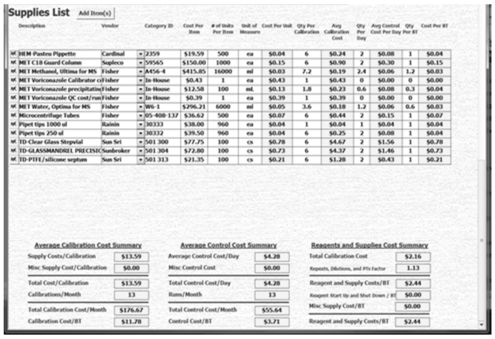
Service Contract Cost

Contract Name	Cost	Years	Cost/Yr
	$63,105.42	1	$63,105.42

Cost/Year	$63,105.42
Total BT/Year	2,227
Cost/BT	$28.34

Put in supply costs



Supplies List Add Item(s)

Description	Vendor	Category ID	Cost Per Item	# of Units Per Item	Unit of Measure	Cost Per Unit	Qty Per Calibration	Avg Calibration Cost	Qty Per Day	Avg Control Cost Per Day	Qty Per BT	Cost Per BT
HEM-Pasteu Pippette	Cardinal	2359	$19.59	500	ea	$0.04	6	$0.24	2	$0.08	1	$0.04
MET C18 Guard Column	Supleco	59565	$150.00	1000	ea	$0.15	6	$0.90	2	$0.30	1	$0.15
MET Methanol, Ultima for MS	Fisher	A456-4	$415.85	16000	ml	$0.03	7.2	$0.19	2.4	$0.06	1.2	$0.03
MET Voriconazole Calibrator c	Fisher	In-House	$0.43	1	ea	$0.43	1	$0.43	0	$0.00	0	$0.00
MET Voriconazole precipitatin	Fisher	In-House	$12.58	100	mL	$0.13	1.8	$0.23	0.6	$0.08	0.3	$0.04
MET Voriconazole QC cost/run	Fisher	In-House	$0.39	1	ea	$0.39	1	$0.39	0	$0.00	0	$0.00
MET Water, Optima for MS	Fisher	W6-1	$296.21	6000	ml	$0.05	3.6	$0.18	1.2	$0.06	0.6	$0.03
Microcentrifuge Tubes	Fisher	05-408-137	$36.62	500	ea	$0.07	6	$0.44	2	$0.15	1	$0.07
Pipet tips 1000 ul	Rainin	30333	$38.00	960	ea	$0.04	1	$0.04	1	$0.04	1	$0.04
Pipet tips 250 ul	Rainin	30332	$39.50	960	ea	$0.04	6	$0.25	2	$0.08	1	$0.04
TD-Clear Glass Stepvial	Sun Sri	501 300	$77.75	100	cs	$0.78	6	$4.67	2	$1.56	1	$0.78
TD-GLASSMANDREL PRECISIC	Sunbroker	501 304	$72.80	100	cs	$0.73	6	$4.37	2	$1.46	1	$0.73
TD-PTFE/silicone septum	Sun Sri	501 313	$21.35	100	cs	$0.21	6	$1.28	2	$0.43	1	$0.21

Average Calibration Cost Summary

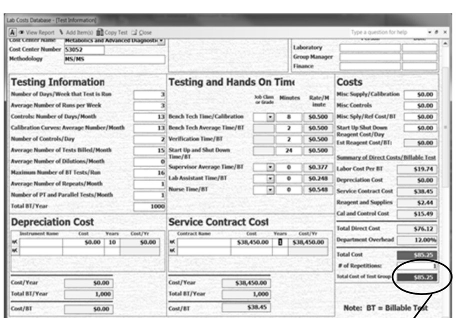
Supply Costs/Calibration	$13.59
Misc Supply Cost/Calibration	$0.00
Total Cost/Calibration	$13.59
Calibrations/Month	13
Total Calibration Cost/Month	$176.67
Calibration Cost/BT	$11.78

Average Control Cost Summary

Average Control Cost/Day	$4.28
Misc Control Cost	$0.00
Total Control Cost/Day	$4.28
Runs/Month	13
Total Control Cost/Month	$55.64
Control Cost/BT	$3.71

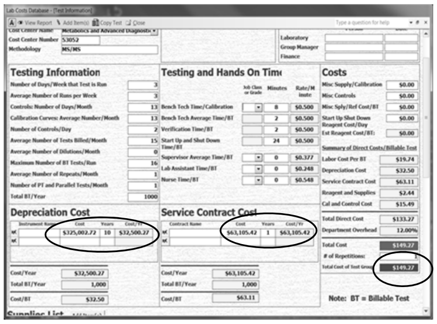
Reagents and Supplies Cost Summary

Total Calibration Cost	$2.16
Repeats, Dilutions, and PTs factor	1.13
Reagent and Supply Costs/BT	$2.44
Reagent Start Up and Shut Down / BT	$0.00
Misc Supply Cost/BT	$0.00
Reagent and Supply Costs/BT	$2.44

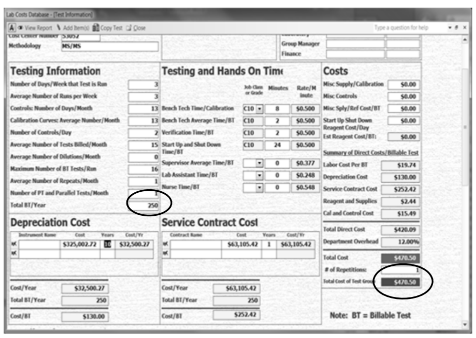


Assay development cost?

Roughly cost/test X number of tests necessary to develop assay



Brand new tandem?



Volume drops?

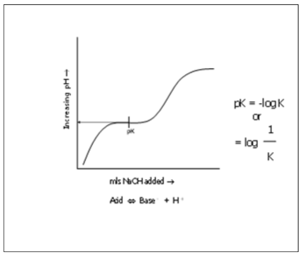
Vori

Conditions	Cost/test	Cost/year	Current cost/year	Savings /year
1000/year and fully depreciated instrument	$85.25	$85,250	$150,000.	**$64,750**
1000 + New instrument	$149.27	$149,270	$150,000	$730
250/year + FD	$214.44	$214,440	$150,000	-$64,400
250/year + new	$470.50	$470,500	$150,000	-$320,500
2000/year + FD	$64.59	$64,590	$150,000	**$85,410**
2000/year + new	$95.73	$95,730	$150,000	**$54,270**

Volumes increased 45%

Buffer

- Solution or compound that minimizes a change in $[H^+]$ (pH) when an acid or base is added.
- A solution has the greatest buffering capacity when the pH is near its pK



Equilibrium constant - K_a

- When a weak acid dissociates it forms an equilibrium between the acid form and the H^+ and base

$$HA \longleftrightarrow A^- + H^+$$

That equilibrium can be described by a constant (K_a) as:

$$K_a = \frac{[H^+]\,[A^-]}{[HA]}$$

80

Henderson-Hasselbalch equation

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

$$-\log [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

Since pH = - log $[H^+]$ and pK_a = - log K_a

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad \begin{matrix} \rightarrow \text{base} \\ \rightarrow \text{acid} \end{matrix}$$

This is the **Henderson-Hasselbalch** equation

81

Use in blood gases

$$pH = pK + \log \frac{[HCO_3^-]}{[CO_2]}$$

$[CO_2] = \alpha \times pCO_2$ where α = a proportionality constant = 0.03 when units are mmol/L

$[HCO_3^-] = tCO_2 - \alpha \times pCO_2$

$$pH = pK + \log \frac{tCO_2 - \alpha \times pCO_2}{\alpha \times pCO_2}$$

or:

$$pH = pK + \log \frac{tCO_2 - 0.03(pCO_2)}{0.03(pCO_2)}$$

If tCO_2 = 26 mM and pCO_2 = 37.7 mm Hg, what is pH?

$$pH = 6.1 + \log \frac{26 - (0.03)(37.7)}{(0.03)(37.7)}$$

$$pH = 6.1 + \log \frac{26 - 1.13}{1.13}$$

$$pH = 6.1 + \log \frac{24.87}{1.13}$$

$$pH = 6.1 + \log 22.01$$

$$pH = 6.1 + 1.34 = 7.44$$

Determine pH of buffers

? pH of a solution of 2 mM acetic acid and 25 mM Na(sodium) Acetate, pK_a = 4.74

$$pH = 4.74 + \log \frac{25 \text{ mM}}{2 \text{ mM}}$$

$= 4.74 + \log 12.5$

$= 4.74 + 1.1 = 5.84$

Acid/Base Ratio needed to make a buffer

A phosphate buffer, pH 5.7, using dibasic and monobasic phosphates, pK = 6.7 (HPO_4^{-2} / $H_2PO_4^{-1}$) (base/acid)

$$5.7 = 6.7 + \log \frac{[HPO_4^{-2}]}{[H_2PO_4^{-1}]}$$

5.7 – 6.7 = log of the ratio

-1 = log of ratio (take antilog of both sides of equation)

0.1 = ratio = 1:10

Make a buffer

A 150 mM citrate buffer, pH 5.2; given: pK = 4.77, citric acid MW = 192.12, Na citrate MW = 215.12

$$pH = pK + \log \frac{[base]}{[acid]} \qquad 5.2 = 4.77 + \log \frac{[base]}{[acid]}$$

$$0.43 = \log \frac{[base]}{[acid]}$$

2.69 = ratio of base to acid

so need: 2.69 moles/L base **:** 1 mole/L acid

Make a buffer

so need: 2.69 moles/L base **:** 1 mole/L acid = total of 3.69 moles/L

$$\frac{1 \text{ mole/L acid}}{3.69 \text{ moles/L total}} = \frac{X}{0.15 \text{ moles/L}}$$

X = 0.04 moles/L acid needed

How much base? 0.15 – 0.04 = 0.11 moles/L base

To make the buffer:

Citric acid: 192.12 g/mole X 0.04 moles/L = X g/L = 7.68 g/L

Citrate: 215.12 g/mole X 0.11 moles/L = X g/L = 23.66 g/L