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11-A	Clinical Bio Chem	7/30/09	

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Jason Largen			Biochem Lab

Title: Experiments in Clinical Biochemistry.

Reference: Experiment 11-A in Modern Exp. Bio Chem 3rd handout from blackboard

Synopsis: In the Clinical setting, it is highly advantageous to be able to quickly assess a patients current level of biological functions. By providing necisary components, enzyme catalyzed reactions can be linked together and used to determine various biochemical levels and functions. Three test reagents are used today to measure levels of glucose, urea nitrogen & Cholesterol in bovine serum. These are then compared to Standards of known values. UV and visible spectro photometry are both used to assess product levels.

Procedure

Blood Glucose

Setup 4 Cuvettes,

#1- 750 mL glucose reagent + 8 mL Standard 1 (5.00mM)

#2 - 750 mL " " + 8 mL Standard 2 (10mM)

#3 - 750 mL " " + 8 mL Standard 3 (20.0mM)

#4 750 mL " " +8 mL bovine serum

mix + let incubate for 15 min

add 375 mL DI water

UV 340nm Reading

blank = DI water

Observations

	A UV 340nm
#1	0.174
#2	0.352
#3	0.536
#4	0.354

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Procedure

Urea nitrogen assay

4 test tubes,

#1 - 1mL Urea nitrogen reagent
#2 - 1mL " " "
#3 1mL " " "
#4 1mL " " "

incubate @ 37°C for 10 min
Zero UV @ Spec. 340 w/ DI water.
Xfer heated tube contents into cuvette, then add
#1 - 10uL Standard 1 (4,0 mM)
#2 - 10uL Std. 2 (8,0 mM)
#3 - 10uL Std 3 (12 mM)
#4 - 10uL bovine Serum

Start timer as soon as Standard or serum added,
read UV340 @ 30s, 60s & 90s

Observations

	30	60	90	Δ30-90
#1	.878	.870	.861	.17
#2*	.905	.894	.882	.23
#3	.878	.845	.835	.43
#4	.915	.913	.909	.07

* reran #2, also ran a 0.0 standard (DI water) Blank.

	30	60	90	Δ30-90
#2	.848	~~.861~~ .840	.829	.19
#0	.902	.901	.902	0.0

the #2 Std, 8,0 mM was not used in determining the line equation, as it was an outlier.

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Procedure

Cholesterol assay

Set up 6 tubes

#1 10 mL DI water 0 mg/dL

#2 10 mL 50 mg/dL Cholesterol Std.

#3 10 mL 100 mg/dL " "

#4 10 mL 200 mg/dL " "

#5 10 mL bovine Serum

#6 ~~10 mL~~ 5 mL bovine Serum + 5 mL DI water.

add 0.5 mL Cholesterol reagent to each. Parafilm & invert mix 3-5 x's

incubate @ 37°C for 10 min.

add 1.0 mL Saline, invert mix.

measure Absorbance @ 510 nm, use DI as blank.

Observations

	A_{510}
#1*	0.050
#2	0.129
#3	0.174
#4	0.268
#5	0.378
#6	0.267

* - this value was dropped from calculations to have more linearity

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Calculations

Glucose

$y = 0,0233x + 0,082$

Serum = 0.354

$X = \frac{0,354 - 0,082}{0,0233}$

$X = 11,67$ mM

Urea Nitrogen

$y = 0,0354x + 0,0114$

Serum = 0,07

$X = \frac{0,07 - 0,0114}{0,0354}$

$X = 1,655$ mM

Cholesterol

$y = 0.0009x + 0.082$

Serum = 0.267 (1:2 dilution)

$X = \frac{0.267 - 0,082}{0,0009}$

$X = (205,6 \text{ mg/dL})(2) = 411,1 \text{ mg/dL}$