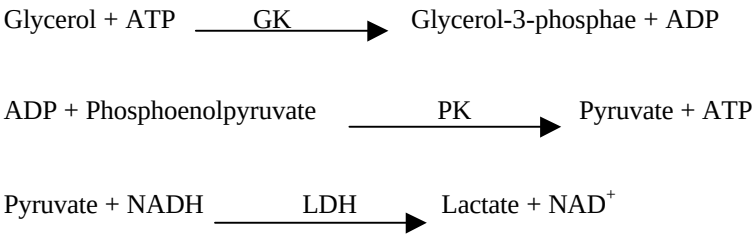
Glycerol Assay

Biochemistry:

Glycerol is phosphorylated to glycerol-3-phosphate by ATP hydrolysis in the glycerkinase reaction. The stiochiometrtic increase in ADP is phosphorylated by phosphoenolpyruvate in the pyruvate kinase reaction to form stoichiometric increases in pyruvate. Pyruvate is then reduced by NADH in the lactate dehydrogenase reaction to form stoichiometric increases in lactate and NAD^+. The concentration of glycerol in the sample is proportional to the decrease in absorbance as NADH is oxidized to NAD^+.



Sample Preparation:

The blood/tissue sample needs to be deproteinized to prevent the slow release of glycerol from blood triglyceride lipolysis. Urine samples do not need prior acid treatment. The deproteinization is best done by a small dilution in 6% PCA. Typically, a threefold dilution works fine, but if glycerol changes induced by exercise are to be accurately measured, a smaller dilution is recommended to ensure as much glycerol in the final sample as possible – perhaps a 1.5 to 2 fold dilution.

Table 1: Assay cocktail ingredients (designed for glycerol ingestion studies).

Compound	Final []	Amount of Compound for given cocktail volume				
		25 mL	***100 mL***	***150 mL***	***200 mL***	***250 mL***
Water		24	96	144	192	140
Imidazole	0.1 mmol/L	0.1702 g	0.6808 g	1.0212 g	1.3616 g	1.7020 g
HCl	0.03 N	0.75 mL	3.0 mL	4.5 mL	6.0 mL	7.5 mL
$MgCl_2$	2.5 mmol/L	0.0119 g	0.0476 g	0.0714 g	0.0952 g	0.1190 g
KCl	50 mmol/L	0.0932 g	0.3728 g	0.5592 g	0.7456 g	0.9320 g
ATP	0.3 mmol/L	0.00381 g	0.01524 g	0.02286 g	0.03048 g	0.03810 g
PEP	0.3 mmol/L	0.00175 g	0.0070 g	0.01053 g	0.0141 g	0.01753 g
NADH	0.3 mmol/L	0.00532 g	0.02128 g	0.03192 g	0.04256 g	0.05320 g
Enzymes						
GK*	0.5 U/mL	50 µL	200 µL	300 µL	400 µL	500 µL
PK#	0.8 U/mL	5 µL	20 µL	30 µL	40 µL	50 µL
LDH^	1.1 U/mL	5 µL	20 µL	30 µL	40 µL	50 µL

* GK is 1,000 U stock diluted in 2 mL = 500 U/mL ; #PK is 2.4 mL of 10,000 U stock = 4167 U/mL ; ^LDH is 4.6 mL of 25,000 U stock = 5435 U/mL

The NADH in this assay should give an absorbance reading between 1-1.5 at 340 nm. Thus, this assay is suited to the measurement of large glycerol concentrations, such as for research of glycerol ingestion. For measurement of lower blood concentrations of glycerol, this assay should be done with each of ATP, PEP and NADH reduced by about half to save chemicals and expense.

Table 2: Stock Sample Locations

Fridge	Freezer	Bench
NADH, KOH, Pyruvate kinase (PK), Lactate dehydrogenase (LDH), glycerol std.	ATP, Phosphoenolpyruvate (PEP), Glycerokinase (GK)	Imidazole, $MgCl_2$, KCl