Hons practical MC 4

GROUPS:

1A
- **Anina Rademeyer**
- **Craig October**
- **Linique Mc Callum**

2B
- **Molatelo Peter**
- **Karli Bothma**
- **Hylton Gibson**

3C
- **Carmen de Villiers**
- **Bianca Nilson**
- **Ankia Visser**

4D
- **Melissa De Lilly**
- **Lee-Maine Spies**
- **Jessica Reid**

5A
- **Elanda Relling**
- **Tyron Nel**
- **Christopher Borrageiro**

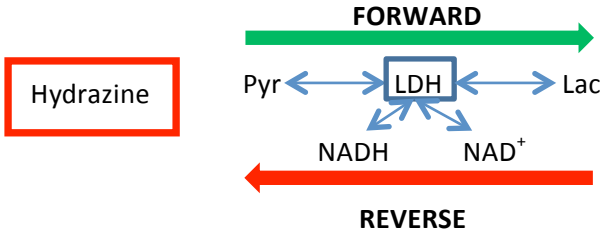
6B
- **Andy van der Berg**
- **Claire du Plessis**
- **Sandisiwe Matyesini**

7C
- **Lieke Dale**
- **Amy Hare**
- **Sarah Kellow-Web**

8D
- **Nicolaas Grobler**
- **Aqilah Benjamin**
- **Aneliswe Ngcobo**

ABOUT THE PRAC:

(1) Characterise the enzyme:



(2) The experiments:

GROUP A
- Reverse direction
- Varying [NAD^+]
- Same [Lac] +[LDH] + [Hydrazine]

GROUP B
- Reverse direction
- Varying [Lac]
- Same [NAD^+] +[LDH] + [Hydrazine]

GROUP C
- Forward direction
- Varying [NADH]
- Same [Pyr] +[LDH]

GROUP D
- Forward direction
- Varying [Pyr]
- Same [NADH] +[LDH]

GROUP A

- Product Inhibition: NADH
- Varying [NADH]
- Same [Lac] +[NAD^+] + [LDH] + [Hydrazine]

GROUP B

- Without Hydrazine
- Varying [Lac]
- Same [NAD^+] +[LDH]

GROUP C

- Product Inhibition: NAD^+
- Varying [NAD^+]
- Same [Pyr] +[NADH]+[LDH]

GROUP D

- Product Inhibition: Lac
- Varying [Lac]
- Same [NADH] + [Pyr]+[LDH]

(3) An enzyme assay:

a- Pipette metabolites into the cuvettes, the reaction is initiated by the addition of the enzyme.

b- Once the enzyme is added measure the absorbance readings for the reaction over a period of 3mins.

c- The types of data we expect to see- Forward reaction: decreasing Abs; Reverse reaction: increasing Abs

(4) What needs to be done:

Create a pipetting schema so that we investigate 10 different concentrations around the Km values. Take the following into consideration:

a- Given :

Km	Stock concentrations
Km(NADH) = 0.05 mM	[NADH] = 1.5 mM
Km(NAD^+) = 0.4 mM	[NAD] = 5 mM
Km(Pyr) = 0.5 mM	[Pyr] = 10 mM
Km(Lac) = 10 mM	[Lac] = 1 M

HYDRAZINE is given at a 10X dilution of bottle concentration

b- Volumes:

- The total cuvette volume is 1 ml and of that you use 10 µl of the enzyme (LDH) and if you are doing **reverse direction experiments (only)** 35 µl is the Hydrazine.
- The other volumes are of the substrate and co-factor which you calculated in the pipetting schema.
- Lastly, the remaining volume to make up this 1 ml volume is done by adding buffer to the cocktail mixture.

c- How to determine the 10 concentrations:

- The range you want to investigate is between: [Km/10; Km) and (Km; 10Km] and the fixed substrates are usually at saturating condition ie. 10xKm. Use Excel to create an incremental pipetting schema.

(5) The Program:

14/03: Wednesday	**15/03 Thursday:**	**16/03 Friday:**
Morning: Practical theory and pipetting schema Afternoon: Group 1-4 (first set of experiments)	Morning: 09H30 Research lecture Mid Morning: Group 5-8 (first set of experiments)	Morning: Group 1-4 (second set of experiments) Afternoon: Group 5-8 (second set of experiments)

19/03 Monday: Redo experiments Afternoon: Data analysis	**20/03 Tuesday:** Morning: Data analysis Afternoon: Seminar	**21/03 Wednesday:** Human rights day
22/03 Thursday: Lecture (9:30) Data analysis (10:30)	**23/03 Friday:** Data analysis	**26/03 Monday:** Hand in report (16:00)