Name: …… Date:…….

GLYCOLYTIC ENZYMES

Notebook

AIM(S) OF THE PRACTICE:

1. ……...

2. ……...

3. ……...

1. INTRODUCTION

Give short definitions about:

Glycolysis:

……

……..……

……..……

……..……

……....……

……

Isozymes:

……

……..……

……..……

……..……

……....……

……

Units of enzyme activity:

……

……

……

……

……

……

Lambert Beer law:

……

……

……

……

……

Colorimetric enzyme activity measurements:

……

……

……

……

……

……

Optical test of Warburg:

……

……

……

……

……

……

Clinical relevance of measuring glycolytic enzyme activity in serum.

……..

……

……..……

……...……

……...……

……....……

…….

2. EXPERIMENTS:

2.1. DETERMINATION OF ALDOLASE ENZYMATIC ACTIVITY (colorimetric assay)

Reaction catalyzed by aldolase (with structures!):

Components of the reaction mixture: (what is the role of these components?):

Serum:

……...……

……....……

TCA:

……...……

……....……

NaOH:

……..……

……..……

DNPH:

……..……

……..……

……

Hydrazine:

……..……

……..……

……

Monoiodine-acetate:

……..……

……..……

……

PROCEDURE (give a short list of what to do –step by step):

1. ……..

2. ……..

3. ……..

4. ……..

5. ……..

6. ……..

MEASURED DATA:

	SERUM #1	**SERUM #2**	**BLANK**
$\mathbf{A_{540}}$			
$\mathbf{A_{minta}}$**-**$\mathbf{A_{blank}}$			
enzymatic activity (nkat/l) *(*A_{sample}*-*A_{blank}*)x574.8*			
enzymatic activity (U/L) *(nkat/l/16.6)*			

CONCLUSION(S):

(Observations /Relevance: write down every important observations, clinical relevance/ diagnostic value of aldolase activity measurement , and evaluate your data comparing them to the given normal value- which of them was likely to come from the diseased patient?)

……

……

……

……

……

……

……

……

……

……

……

……

……

2.2. DETERMINATION OF LDH ENZYMATIC ACTIVITY (Warburg optical test)

Reaction catalyzed by lactate dehydrogenase (with stuctures!):

How can be the equilibrium shifted towards pyruvate production?

……..……

……..……

……..……

Components of the reaction mixture: (what is the role of these components?):

Lactate: ……..

……..

……..

Hydrazine: ……..

……..

……..

……..

NAD: ……..

……..

……..

PROCEDURE (give a short list of what to do –step by step):

1. ……..

2. ……..

3. ……..

4. ……..

MEASURED DATA:

TIME (min)	SERUM #1 A_{366}	SERUM #2 A_{366}
0		
0.5		
1.0		
1.5		

TIME (min)	SERUM #1 A_{366}	SERUM #2 A_{366}
2.0		
2.5		
3.0		

GRAPH:

Plot the normal and pathological line on the same graph!

Y axis: absorbance values (A_{366}); X axis: time (min)

Draw a line, and calculate the slope! If the points give a curve instead of straight line, draw a straight line based an the very first points which belongs to the linear part of the curve.

Determine the slope of the lines! **slope=ΔA/Δt**

SERUM #1:

……

SERUM 2#:

……

CALCULATE the LDH activity in nkat/l **slope x 50505**

SERUM #1:

…… nkat/l

SERUM #2:

…… nkat/l

CONCLUSION(S):

Evaluation of data / Observations /Relevance: write down every important observations, clinical relevance/diagnostic value of aldolase activity measurement , and evaluate your data comparing them to the given normal value- which of them was likely to come from the diseased patient?

Normal value: ……

……

……

……

……

……

……

……

……

2.3. SEPARATION LDH ISOENZYMES BY ELECTROPHORESIS (DEMONSTRATION)

Isoforms of LDH (types, tissues, organ distribution, composition, structure):

……

……

……

……

……

……

……

……

Membrane electrophoresis (principles of the method):

……

……

……

……

……

……

……

……

Activity-staning (principles of the method, composition of the gel):

……

……

……

……

……

……

……

……

PROCEDURE (give a short list of what to do –step by step):

1. ……..

2. ……..

3. ……..

4. ……..

5. ……..

6. ……..

RESULTS:

Evaluate your data- by copying the pattern shown up on the cellulose acetate membrane, and identifying the bands. Do not forget to indicate samples, cathode, anode, start line, direction of the current, LDH isoforms!)

Order of the samples (from the left to the right):

1. …….

2. …….

3. …….

4 tracer ……..……..

CONCLUSION(S):

Write down what kind of isoforms do you recognise in the different tissue samples! Is it that what you expected? use table I in BPG!

Sceletal muscle:

……

……..……

Lung:

……

……

Cardiac muscle:

……

……

LDH 1-4 isoformes move towards the positive pole (anode) while LDH5 towards the negative (catode). Why? is there any diagnostic value of the determination of LDH isoform patterns in serum?

……

……

……

……