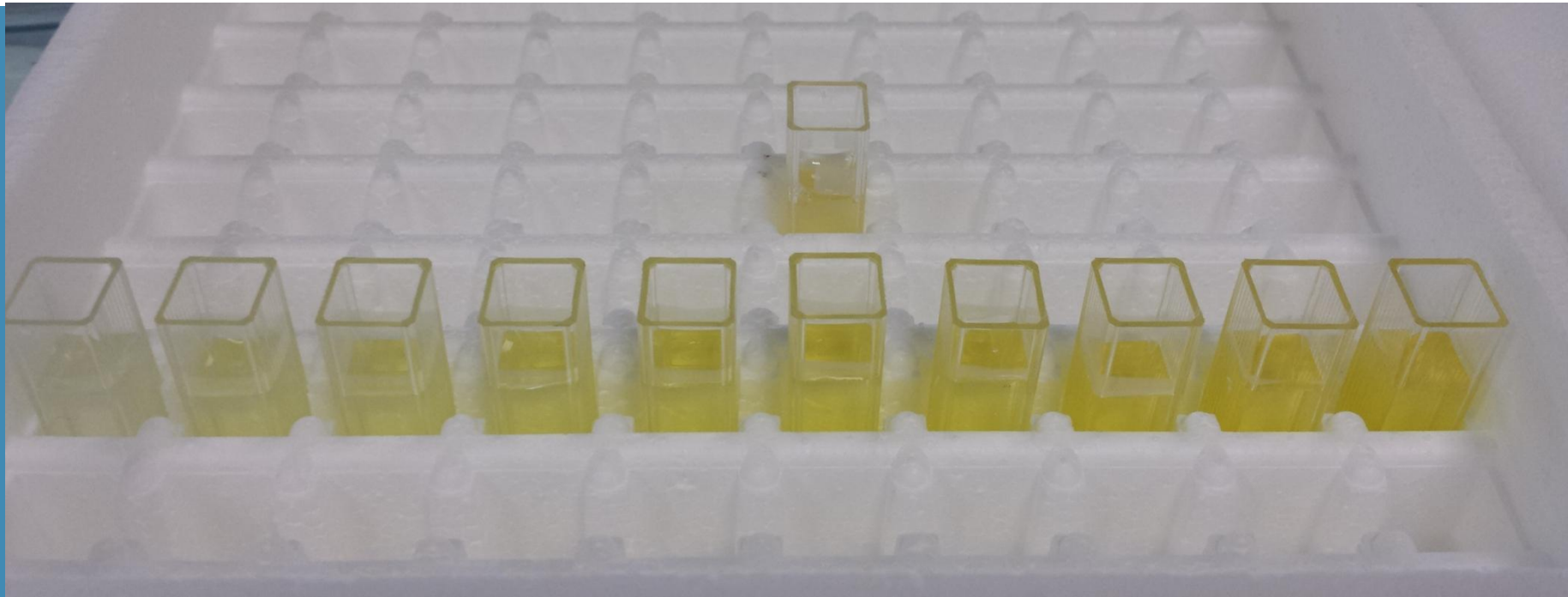
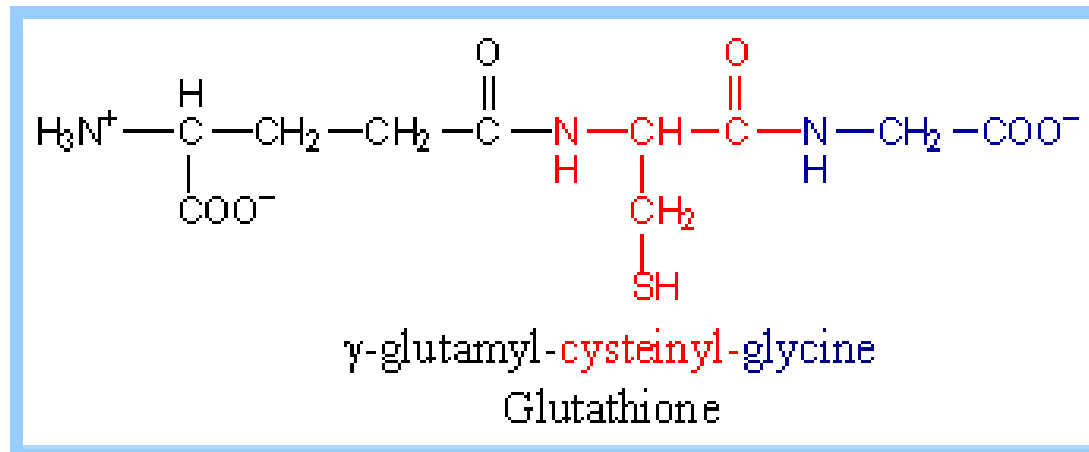


ESTIMATION OF GLUTATHIONE IN PLASMA



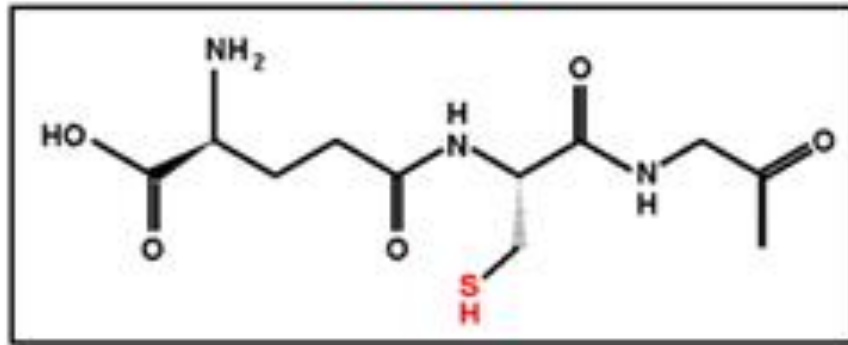
Glutathione (GSH) is produced naturally **by the liver**, GSH is the most abundant **intracellular** thiol an important antioxidant preventing damage to important cellular components caused by reactive oxygen species such as **free radicals** and **peroxides**.



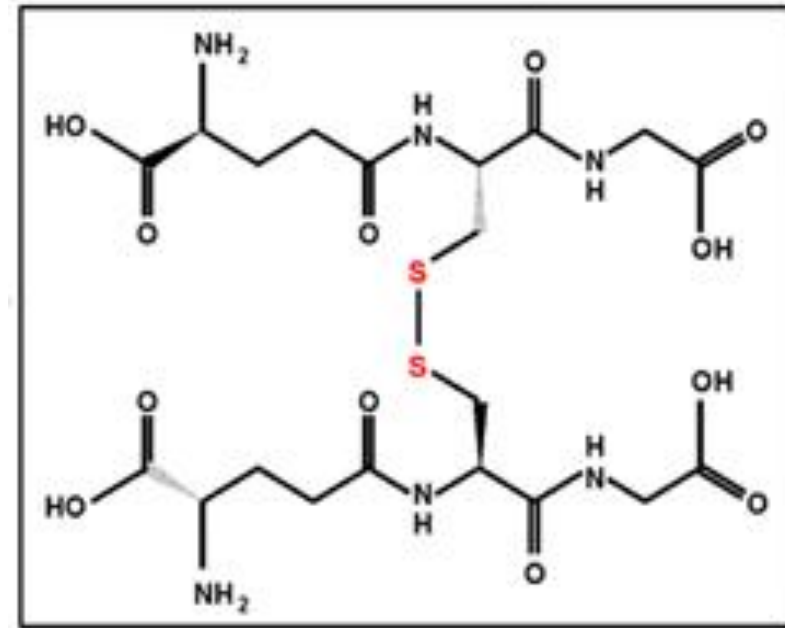
Glutathione structure :Tri peptide with a γ(gamma) -peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine (which is attached by normal peptide linkage to a glycine).

THERE ARE TWO FORMS OF GLUTATHIONE

GSH



Reduced form(GSH)



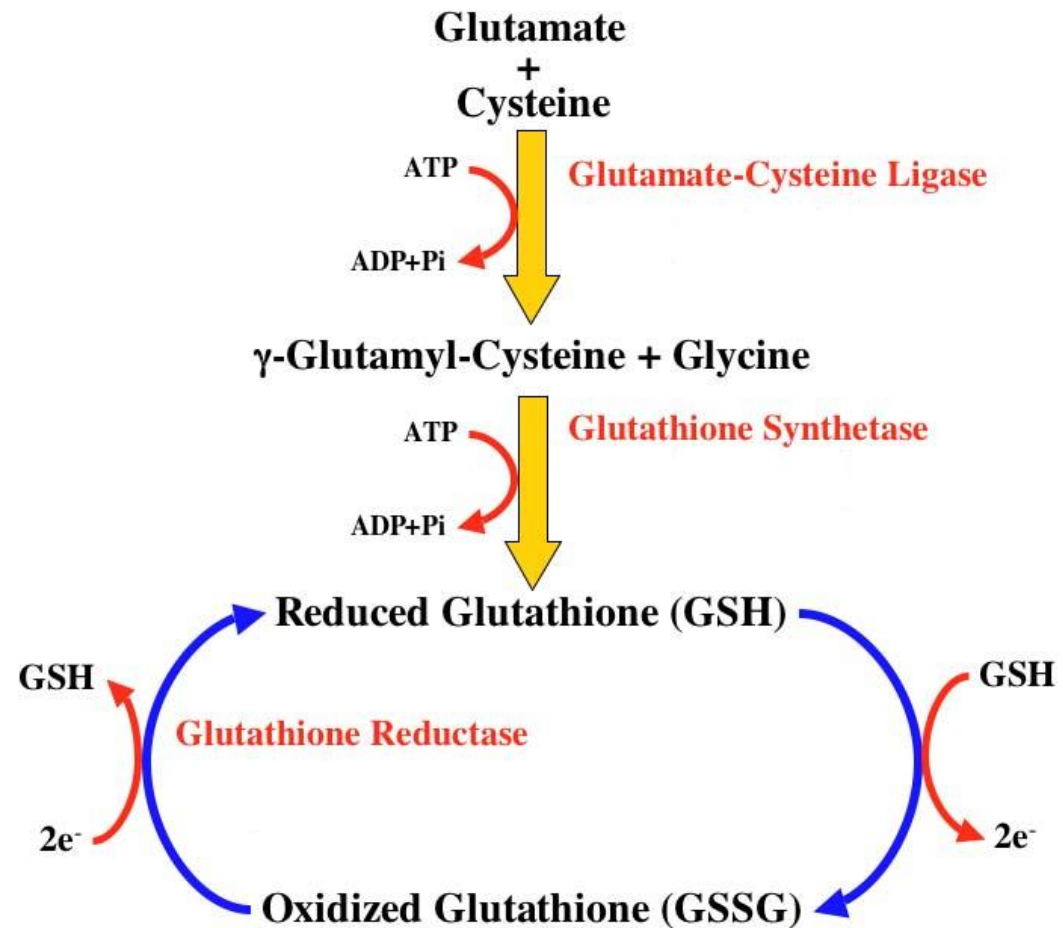
GSSG

Oxidized form (GSSG).

In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress.

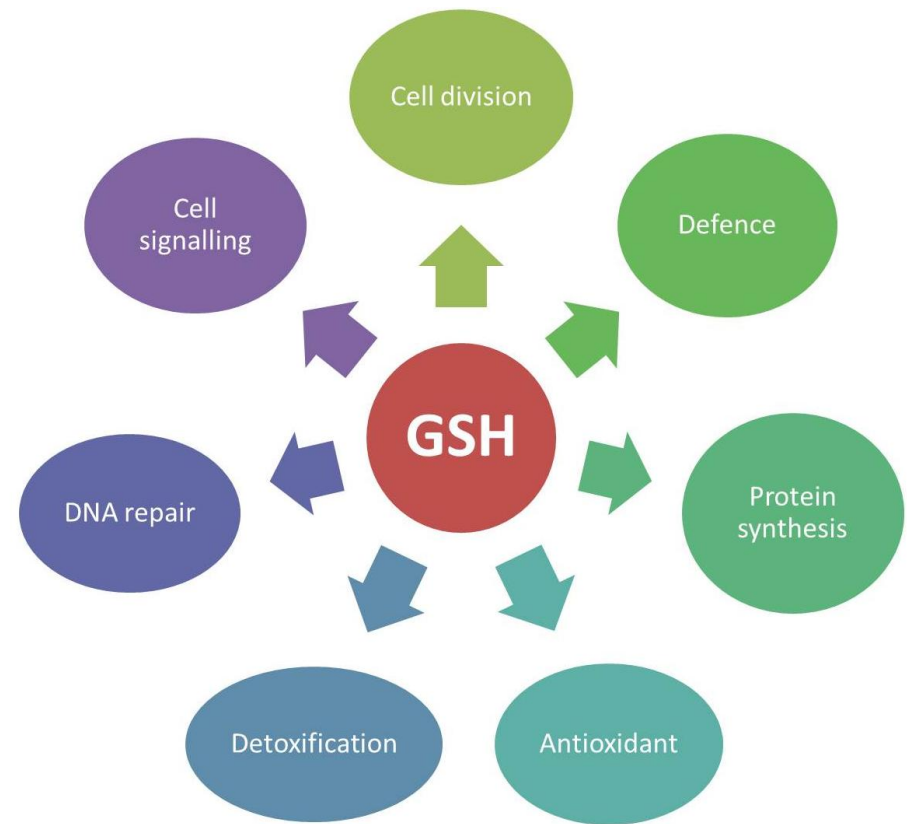
GLUTATHIONE BIOSYNTHESIS AND REGENERATION

Biosynthesis and Regeneration



What is the major roles of glutathione in the body?

1. maintaining the **sulfhydryl** groups of proteins in the **reduced** state
2. **Antioxidant** system of most **aerobic cells** ;remove toxic peroxides formed in the normal course of growth and metabolism under aerobic conditions.
3. GSH plays a critical role as a **coenzyme with a variety of enzymes** including, glutathione peroxidase, glutathione S-transferase and thioltransferase.
4. Play a central role in **metabolic pathways** ;drug metabolism, calcium metabolism, the g-glutamyl cycle, blood platelet and membrane functions.
5. **detoxification** of xenobiotics



REGULATION

- Glutathione is a tightly regulated intracellular constituent; **limited** in its production by negative feedback inhibition of its own synthesis through the enzyme γ -**glutamylcysteine synthetase**, thus greatly minimizing any possibility of overdose.

WHAT IS THE ABNORMALITIES OF GLUTATHIONE MEAN?

- **Low glutathione** is commonly observed in
- Cancer
- HIV/AIDS
- trauma
- burns
- athletic overtraining
- high oxidative stress.

Physiological values of intracellular GSH generally range from 1 to 10 mM.

A deficiency in GSH can result in increased susceptibility to cell lysis due to lipid peroxidation

Normal range : 4-10mg/dl

GLUTATHIONE FOR TREATING :

INTRAVENOUS, Reducing side effects of chemotherapy treatments for cancer and xenobiotic overload c.

TAKEN BY MOUTH for maintaining the body's defense system (immune system) and fighting metal and drug poisoning.

- Cancer.
- Heart disease.
- High cholesterol levels.
- diseases that weaken the body's defense system AIDS ,Alzheimer's disease, and Parkinson's disease.

INHALED

for treating lung diseases, cystic fibrosis

Objective

I-To estimate the amount of glutathione in Plasma by drawing standard Curve of Glutathione using known concentration of glutathione by spectrophotometric technique.

EXPERIMENTAL DETERMINATION OF GLUTATHIONE REDUCTASE ENZYME ACTIVITY

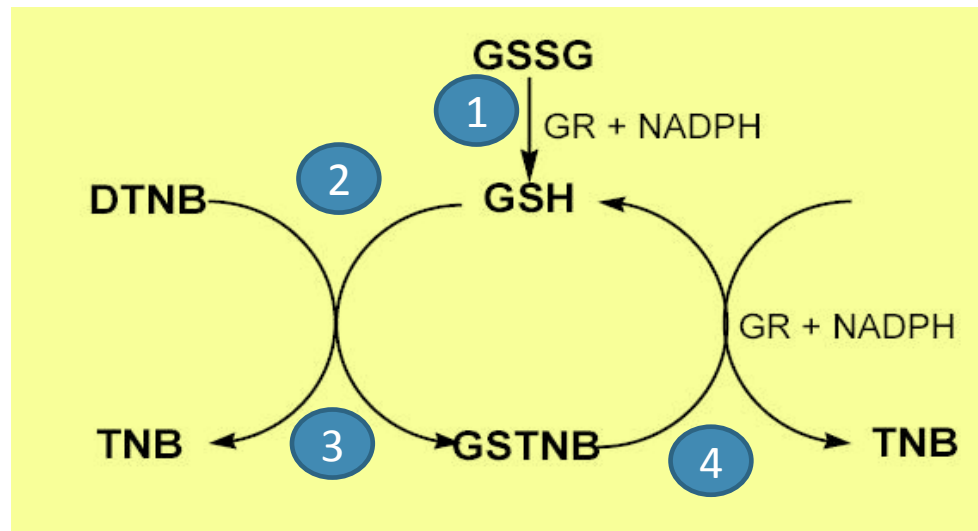
First method is **UV assay** this deals with the oxidation of **NADPH** so, the activity can spectrometrically be observed at 340 nm which can be calculated by molar extinction coefficient (E_o) $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ of NADPH49.

Second one, is **colorimetric quantification** method can be **measured by formation of GS-TNB complex from DTNB** (5, 5' dithiobis (2-nitrobenzoic acid)) develops yellow color because of the DTNB reduction. The GR activity can be measured by visible range at 412 nm, the total activity can be calculated from E_o : $14.15 \text{ mM}^{-1} \text{ cm}^{-1}$ of TNB85.

PRINCIPLE:

- The reagent, 5-5'-dithiobis[2-nitrobenzoic acid] (DTNB, Ellman's Reagent) reacts with GSH to form the 412 nm chromophore, 5-thionitrobenzoic acid (TNB) and GS-TNB.
- The GS-TNB is subsequently reduced by glutathione reductase and β -nicotinamide adenine dinucleotide phosphate (NADPH), releasing a second TNB molecule and recycling the GSH; thus amplifying the response.

Any oxidized (GSSG) initially present in the reaction mixture or formed from the mixed disulfide reaction of GSH with GS-TNB is rapidly reduced to GSH



The process cycles continuously until all of the DTNB is reduced or NADPH is consumed.

MATERIALS AND EQUIPMENT

- Glacial metaphosphoric acid,
- Disodium ethylenediamine tetra acetic acid(EDTA),
- Sodium chloride, sodium monohydrogen phosphate(Na_2HPO_4),
- 5,5-dithiobis-2-nitrobenzoic acid(DTNB), sodium citrate and glutathione
- Spectrophotometer
- Glass test tubes.
- Heparin coated test tubes
- Adjustable pipettes with disposable tips.
- Glass test tubes and vortex.
- Water bath kept within 22 - 37°C temperature range.

METHOD

Part I: Preparation of serial GSH concentration :

Tube	Glutathione Stock (10mg/dl)	Na ₂ PO ₄ (0.3M) Phosphatesolution (ml)	DTNB reagent
Blank	0	3	0.25 ml
1	0.1	2.9	0.25 ml
2	0.2	2.8	0.25 ml
3	0.3	2.7	0.25 ml
4	0.4	2.6	0.25 ml
5	0.5	2.5	0.25 ml
6	0.6	2.4	0.25 ml
7	0.7	2.3	0.25 ml
8	0.8	2.2	0.25 ml
9	0.9	2.1	0.25 ml
10	1	2.0	0.25 ml
Serum sample	0.5	2.5	0.25 ml

Total volume in each tube is 3 ml and the concentration of Glutathione Stock (10mg/dl)

1. Incubate all tubes 10 min at 37°C in water bath , the read the Absorbance at 412 nm.
2. Record your result

Part II: Preparation of Blood Sample for GSH determination:

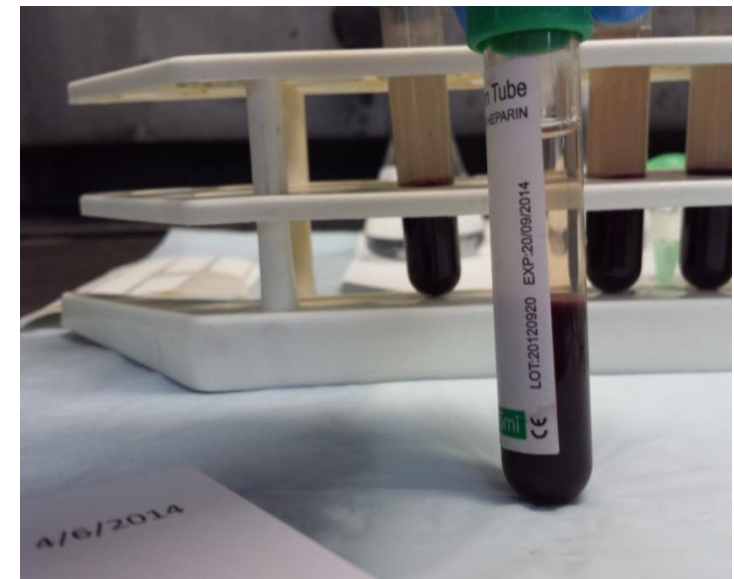
- 1-Collect the blood in heparinized test tubes. Picture (1)
 - 2-Centrifuge blood at 600xg at 4°C for 10 minutes. Picture (2)
 - 3-The pellet contains the red blood cells and the supernatant is the plasma fraction . Picture (3)
- Keep the supernatant (plasma) for glutathione assay. Discard the precipitate(erythrocytes).



Picture (1)



Picture (2)



Picture (3)

Part II: Preparation of Blood Sample for GSH determination:

5-Add 2.5ml of Na_2PO_4 (0.3M) “phosphate buffer” to 0.5ml of above supernatant.

6-Add 0.25ml of DTNB-Reagent

7-Incubate the above mixture for 10min at 37°C.

8-Read the absorbance at 412nm using spectrophotometer.

9- determine the concentration of by the standard curve

10- Then calculate the total amount of glutathion if you know That the total plasma volume of the sample was = 12ml

RESULTS AND CALCULATIONS: :

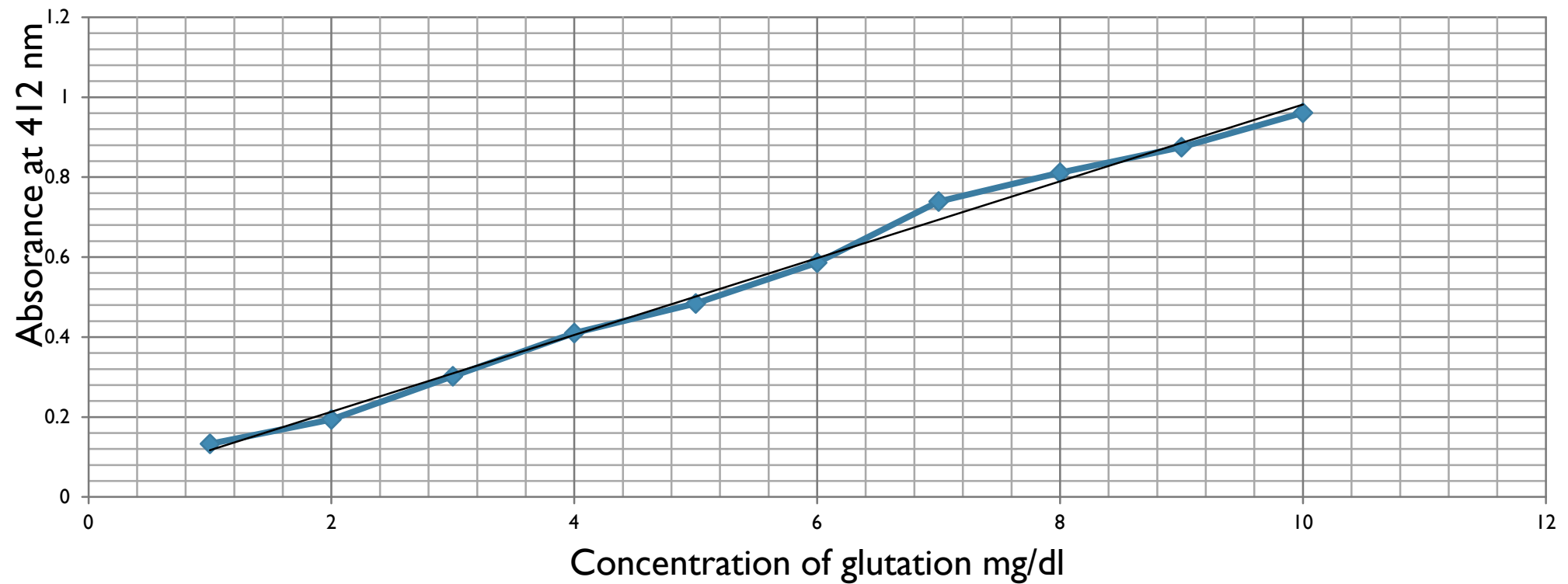
Tube	Concentration of glutathione mg/dl	Absorance at 412 nm
Blank	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Serum sample	From the curve	

calculate the concentration of glutathione in each tube

RESULTS:

draw the standard curve.

Standard curve of glutathione with DNTB reagent



DISCUSSION:

- **From the curve determined the concentration of Glutathione in your sample and compare it to the normal range. And discuss the reasons of abnormalities .**

REFERENCES

- http://www.csulb.edu/~zedmason/students/current/research/rhondamoeller/moeller_introduction.html
- <https://www.funakoshi.co.jp/data/datasheet/NWS/NWK-GSH01.pdf>
- **MINI-REVIEW: METABOLIC FUNCTIONS AND MOLECULAR STRUCTURE OF GLUTATHIONE REDUCTASE** Chandra M.
Phytomatics Laboratory, Department of Bioinformatics, Bharathiar University,
Coimbatore- 641046, Tamil Nadu, India