Estimation of Glutathione in Plasma

- Objectives:

- ✓To draw the standard Curve of Glutathione by given known amount of glutathione assay procedure using spectrophotometric technique.
- ✓To estimate the amount of glutathione in plasma sample.

-Introduction:

- **Glutathione** (gamma-glutamylcysteinylglycine or GSH)

is a naturally occurring **tripeptide**.

- It has nucleophilic and reducing properties.

- **Glutathione function:**

1- Play a central role in metabolic pathways.

2- Play as antioxidant system of most aerobic cells.

3 - GSH plays a critical role as **a coenzyme** with a variety of enzymes including, **glutathione peroxidase, glutathione S-transferase and thiol transferase**.

4- GSH also plays major roles in drug metabolism, calcium metabolism, the g-glutamyl cycle, blood platelet and membrane functions.

- **In addition**, **GSH** is crucial to a variety of life processes, including

1. The detoxification of xenobiotics.
2. Maintenance of the SH level of proteins.
3. Thiol-disulfide exchange.
4. Removal of hydroperoxides and free radicals.
5. Amino acid transport across membranes.

- Physiological values of intracellular GSH generally range from 1 to 10 mM.
- **Glutathione's three major roles in the body are:**

1- Anti-oxidant

2- Blood Booster

3- Cell Detoxifier

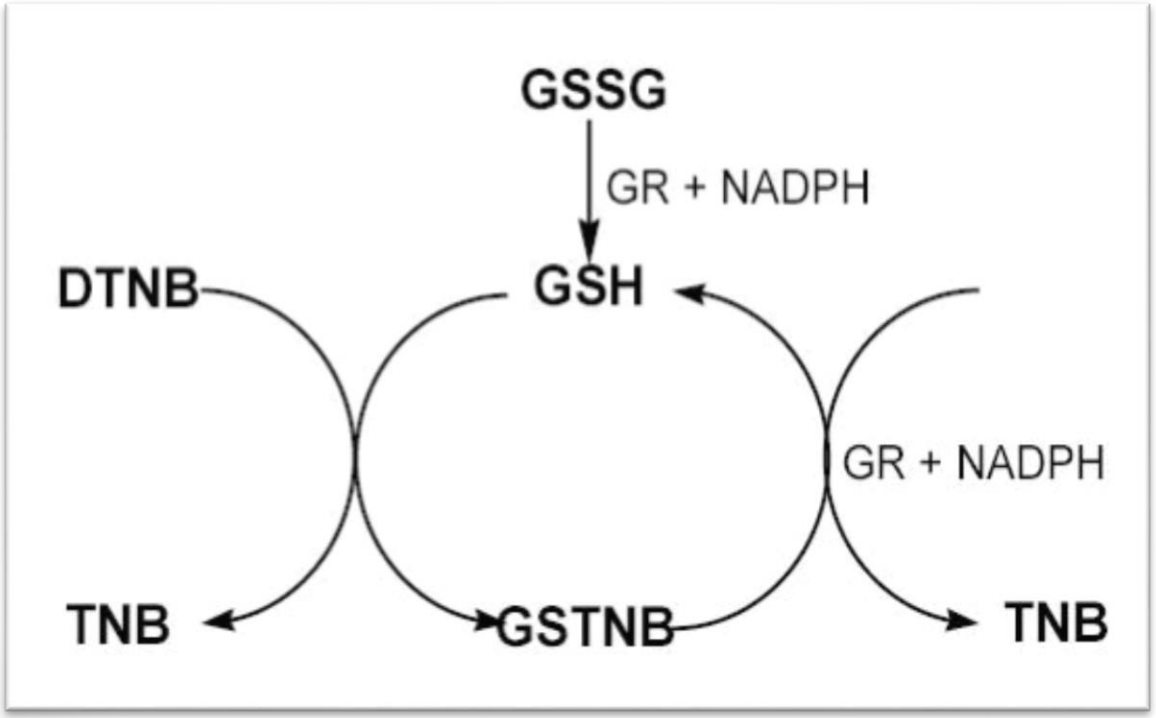
Glutathione deficiencies have been linked to many forms of **cancer**.

Assay Principle

- The principle of the assay is based on **the oxidation of the reduced form of glutathione** by the aromatic disulphide compound and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) to form GSSG and the aromatic thiol,5thio-2nitrobenzoic acid (TNB).

  The yellow color formed is measured **at 412nm** and is proportional to the amount of glutathione present in the sample.

- The enzymatic recycling method for quantitation of GSH and/or GSSG. **GSSG, oxidized glutathione;; GSH, reduced glutathione; GR, glutathione reductase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TNB, 5-thio-2-nitrobenzoic acid; GSTNB, the disulfide product of reaction of GSH with DTNB.**



- Method:

Preparation of serial GSH concentration

Tube No.	GSH stock Solution (ml)	Phosphate Solution (ml)	DTNB (ml)	Total Volume (ml)	GSH Concentration (mg/dl)	GSH Concentration (µg/ml)
1	0.6	2.15	0.25	3	2	20
2	1.2	1.55	0.25	3	4	40
3	1.8	0.95	0.25	3	6	60
4	2.4	0.35	0.25	3	8	80
5	3	0	0.25	3	10	100
Blank	0	0.6	0.25	3	0	0
Sample 1	0.3	2	0.25	3		

- Tubes from 1 to 5- Take 0.5 ml of solution+ 2ml of Ph Solution+ + 0.25 DTNB+ 0.25 H2O.

- For sample: **Make up the volume to 3ml by adding 0.45 ml H2O.**

- For Blank: **Make up the volume to 3ml by adding 2.15 ml H2O.**

↓

Water bath at 37 ^{o}C for 10 min → Read absorbance at 412 nm

- *Glutathione standard curve data:*

Tube No.	GSH concentration (µg/ml)	Absorbance At 412 nm
1	20	
2	40	
3	60	
4	80	
5	100	
Blank	0	
Sample 1		

- **How to convert concentration unit from mg/dl to µg/ml ?**
- 2 mg/dl to µg/ml ⟶ 2 x 1000/100= 20 µg/ml

Preparation of Blood Sample for GSH determination.

- *Best results are obtained with fresh blood samples*

1- Collect the blood in **heparin test tubes.**

- Immediately **shake the tubes** and keep the blood at $4^{o}C$.
- **Centrifuge at least 5 ml of whole blood at 600g at 4°C for 10 minutes.**

3. The **pellet contains the red blood cells** and the **supernatant is the plasma** fraction.

4. **Keep the supernatant (plasma) for glutathione assay. Discard the precipitate (erythrocytes).**

5. Take 0.2 ml of plasma supernatant +1.8ml of deionized distilled water + 0.3ml of precipitating reagent .

6. **Centrifuge at 1200Xg for 10 min.**

7. Take 0.3ml of above supernatant+ 2ml of Na2PO4(0.3M) +0.25ml of DTNB-Reagent .

- **Make up the volume to 3ml with distilled water 0.45 ml H2O.**

8- **Incubate the above mixture for 10min in water bath 37^{o}C.**

9- **Read the absorbance at 412nm using spectrophotometer.**

- **Calculation of glutathione Concentration:**

✓ The **glutathione concentration** in the sample was calculated by **plotting its absorbance on the standard curve** and expressed as **µg/ml** of the plasma.

✓ **Total volume of extracted plasma is 3 ml, so calculate the concentration in 3 ml of plasma.**

✓ **Calculate sample concentration of the sum GSH and GSSG in mg by converting µg to mg.**

- **Normal range:**

- **GSH and GSSG concentration** = 3.8-5.5 **µmol/L =** 3.344-4.84 **mg**

- 1 **µmol/L = 0.88 mg**