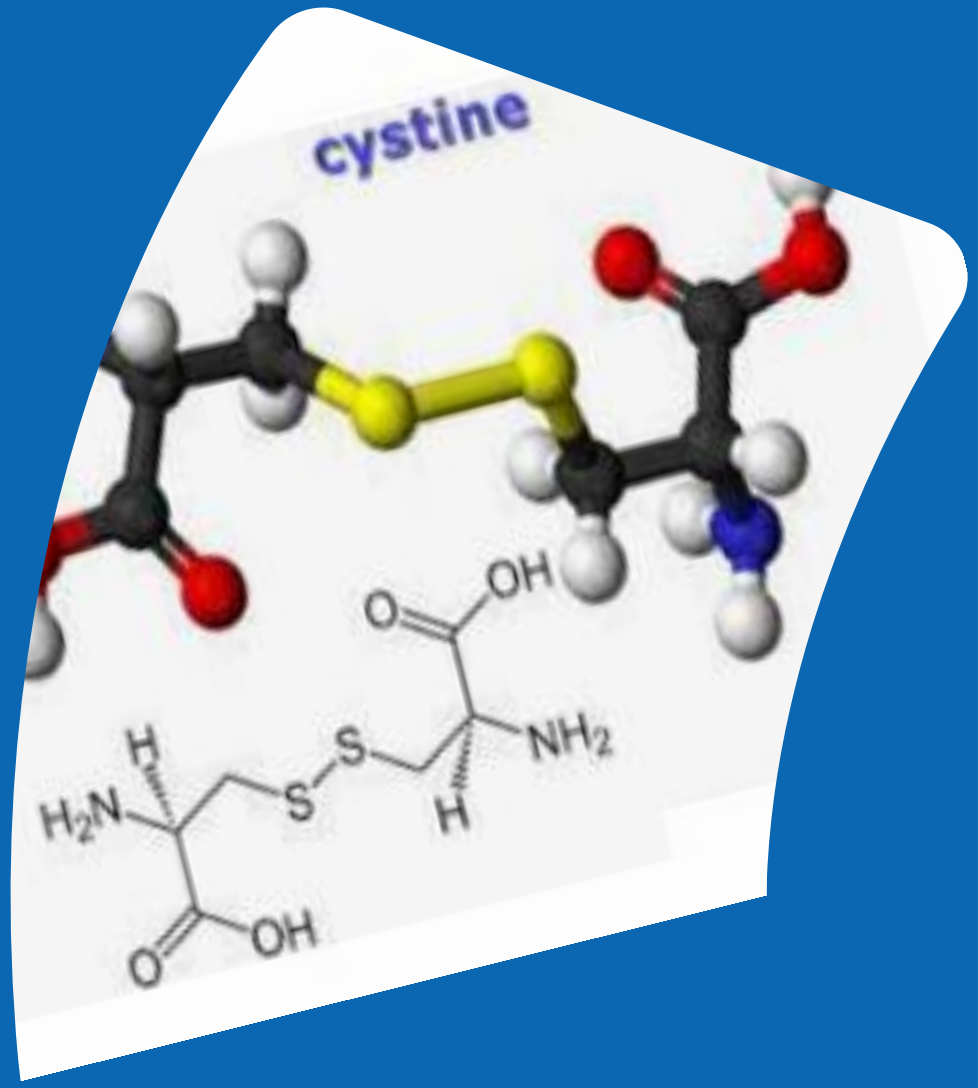
Cliniques universitaires SAINT-LUC UCLouvain BRUXELLES

# Monitoring of cysteamine treatment: laboratory perspective

Sandrine MARIE – Cliniques Saint-Luc

08-07-2022 Leuven



# Introduction

- Cystinosis : accumulation of cystine in the cells (lysosomes, cellular organites)
- Monitoring : intraleucocyte cystine assay every 3 month
- Rare disease plan : CUSL, reference laboratory for IL cystine assay in Belgium
- Cost of the analysis is covered by Sciensano
- A calendar with 4 dates (+1) / year is proposed for patients follow-up

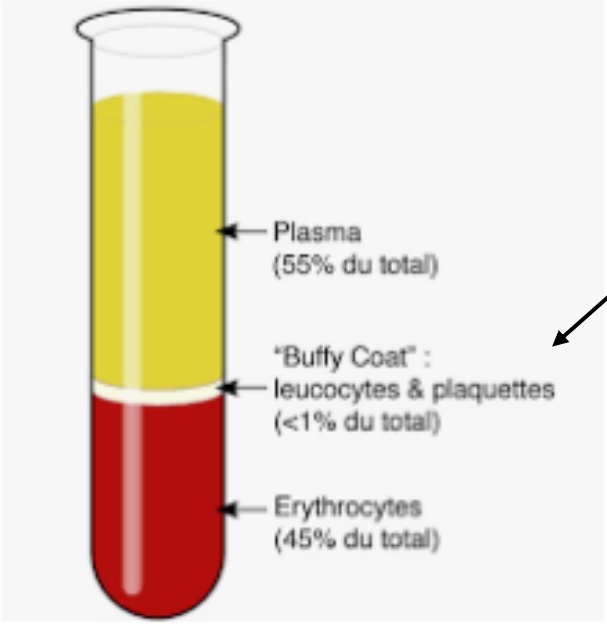
# Intraleucocyte cystine assay

1. Cell isolation : Leucocytes versus granulocytes
2. Cell extract : Separation of proteins and soluble extract
3. Cystine assay : Mass spectrometry (LC-MSMS)
4. Results : ½ cystine /mg protein

# 1. Cell isolation

## Mixed leucocytes versus granulocytes

- Blood collection : 7-10 ml veinous blood  in ACD tubes (6h after Cystagon / 12h30 after Procysbi)
- Cell isolation within 24h after collection (48h max  cf HAS France)




**Isolation of mixed Leucocytes** (plasma and RBC removal) :

| | |
|---|---|
| Granulocytes (rich in lysosomes) | 60-65% |
| Agranulocytes (poor in lysosomes) | 35-40% |

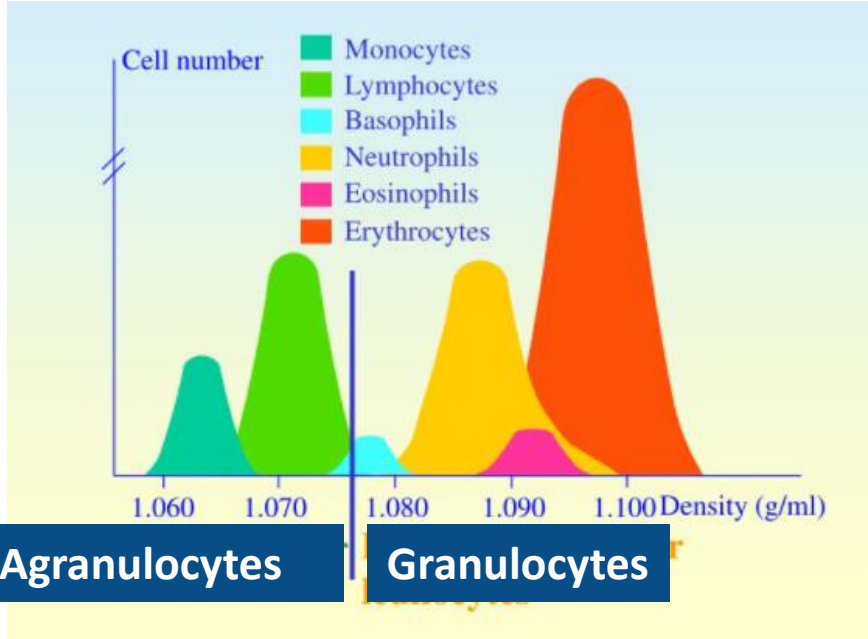
# (1) Cell isolation - *CUSL*

## Isolation of granulocytes : (1) use of cell density difference

- Leucocytes are mixed with a Dextran solution
- Low speed centrifugation

**Suspension of Agranulocytes**

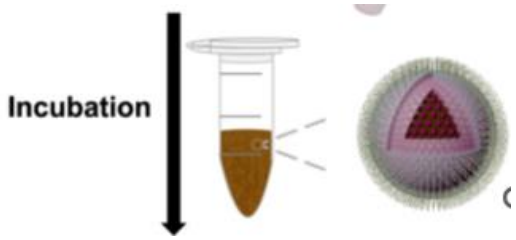
**Pellet of Granulocytes**



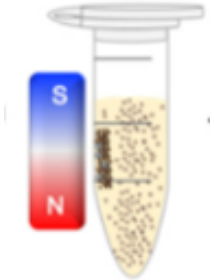
# (1) Cell isolation – *Nijmegen*

isolation of granulocytes : (2) use of magnetic beads

- Antibodies specific to granulocytes membrane, linked to magnetic beads
- The antibody « capture » granulocytes




- Separation with a magnet

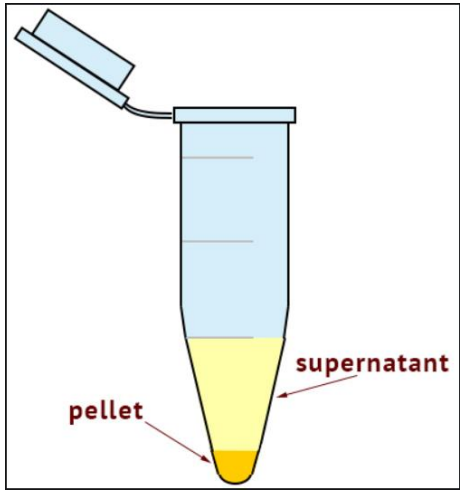



**Granulocytes**

# 2. Cell extract

## Lysis of granulocytes

- The granulocytes are lysed with an acidic solution (Sulfosalisilic acid)
- The cell lysate extract is centrifuged to separate insoluble proteins / soluble supernatant containing cystine



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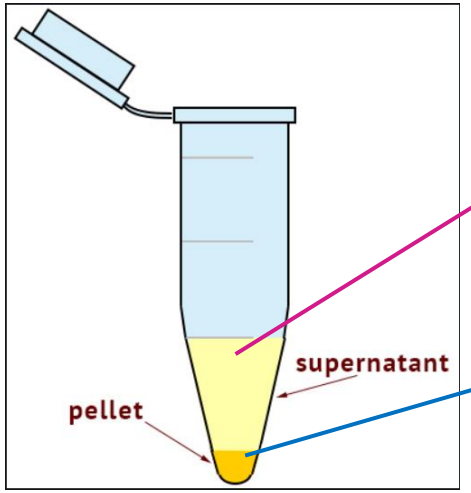



***Cystine assay***

***Protein assay***

# 3. Cystine assay

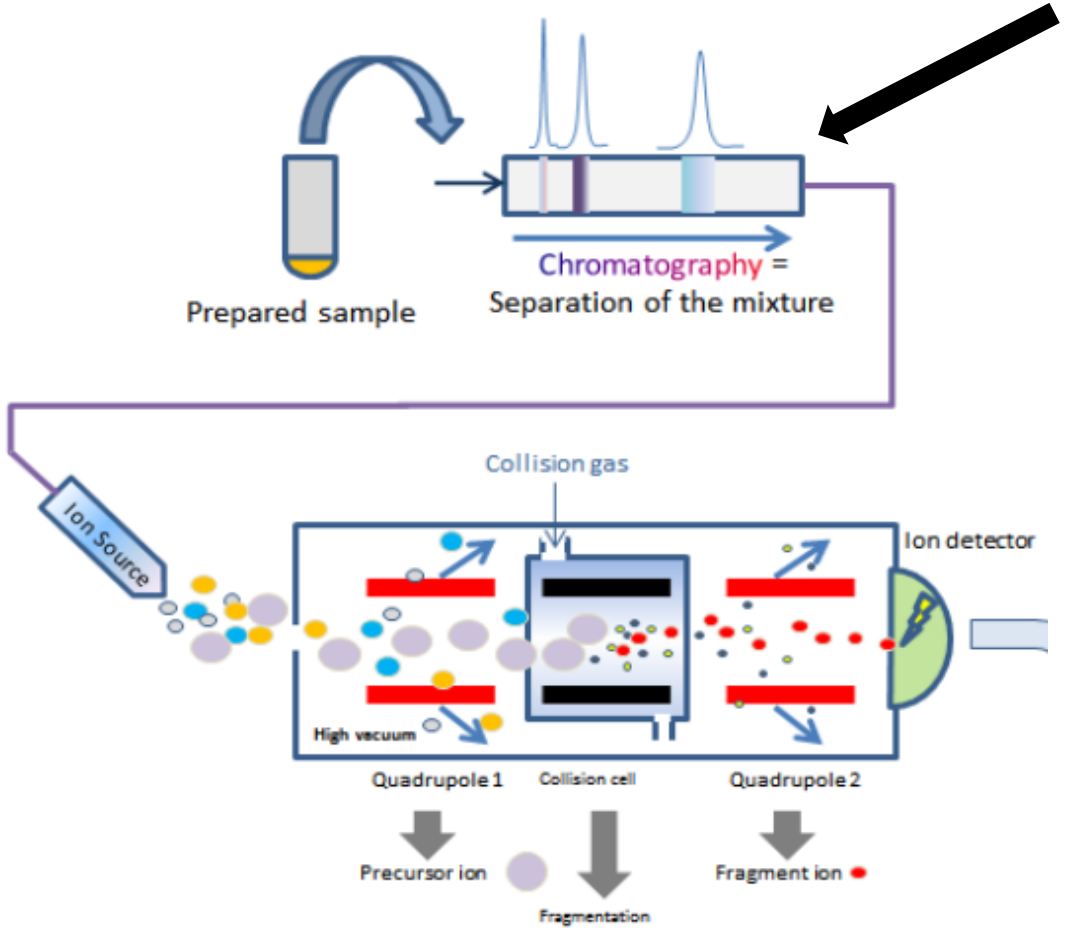
## Mass spectrometry (LC-MSMS)

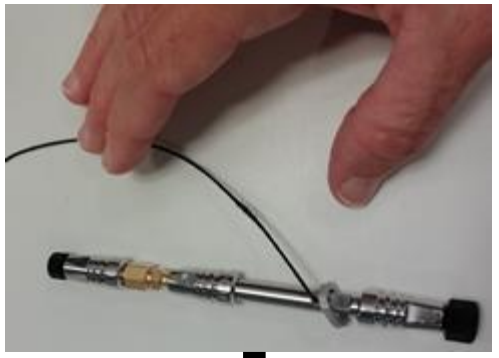
1 – Separation of molecules on a column, by Liquid Chromatography (LC)

2- Detection (and quantification) of cystine by a Mass Spectrometer

(able to separate and identify the molecules according to their mass)

# Mass spectrometry (LC-MSMS)



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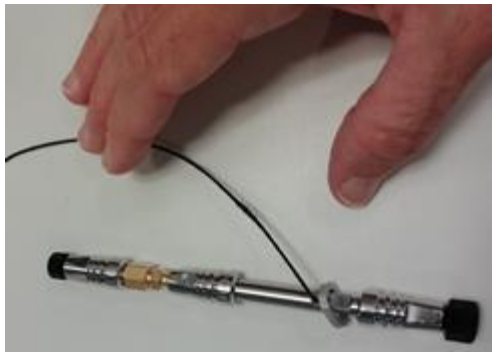
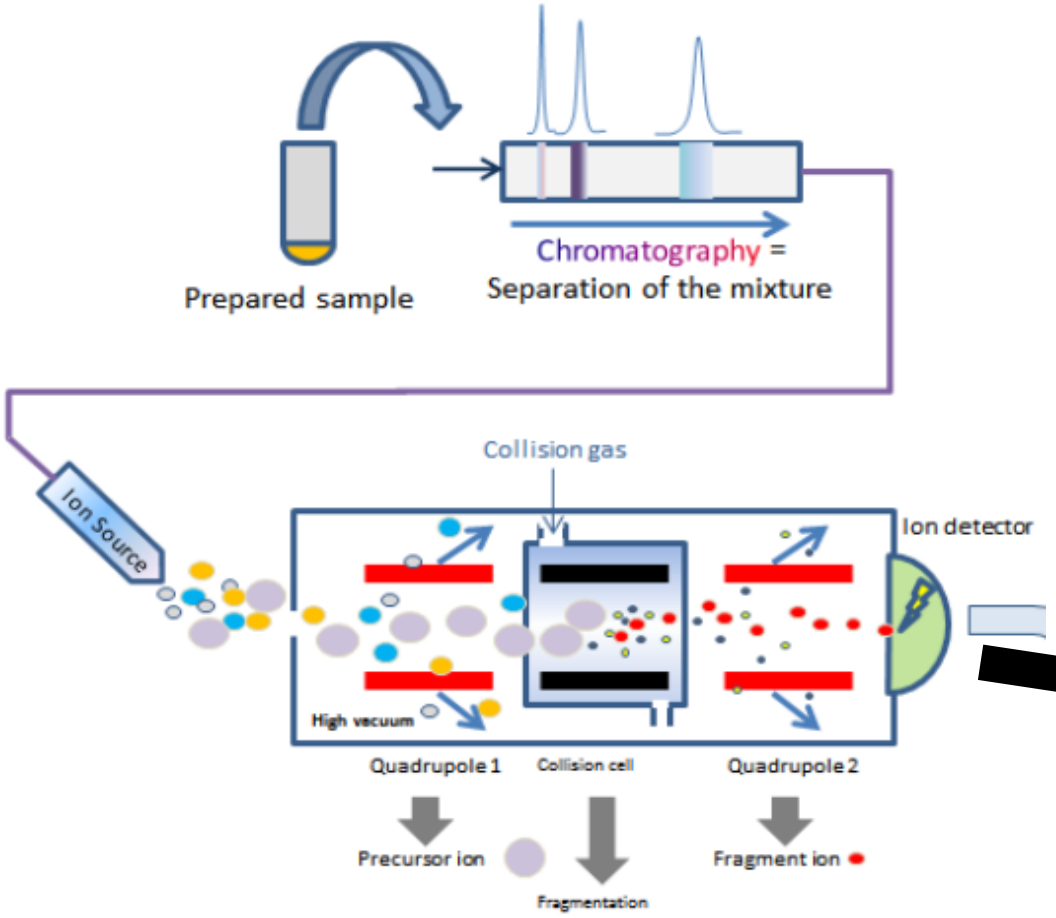
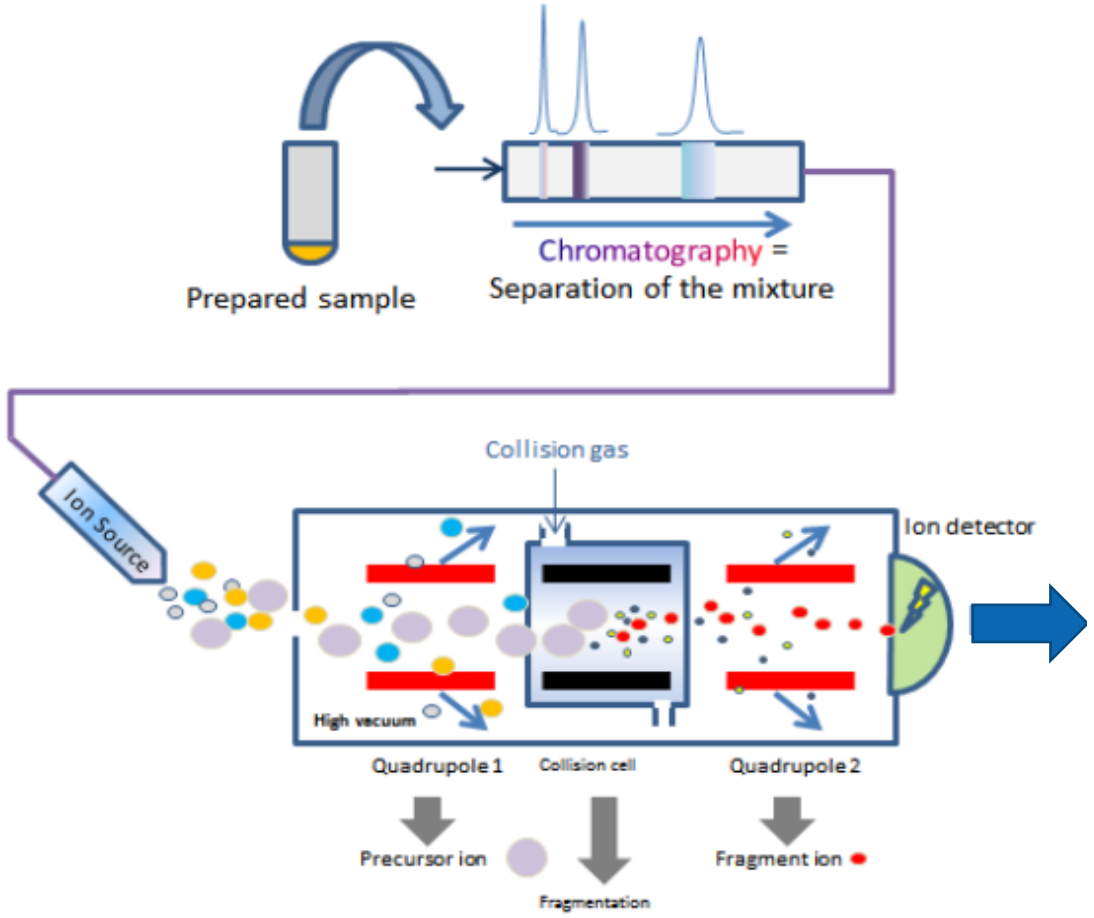





# Mass spectrometry (LC-MSMS)




## Signal : peak area

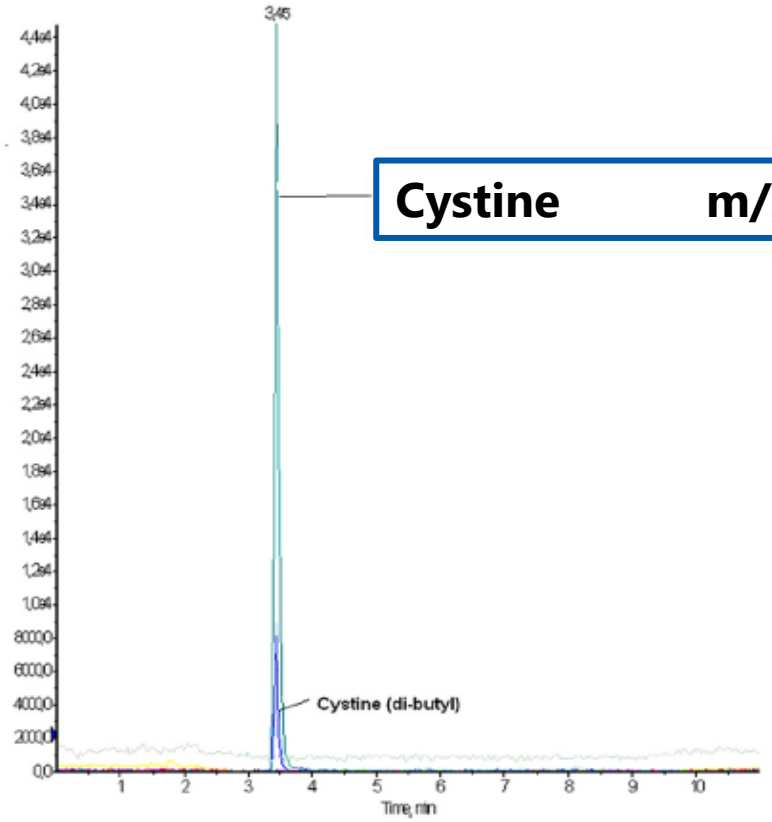


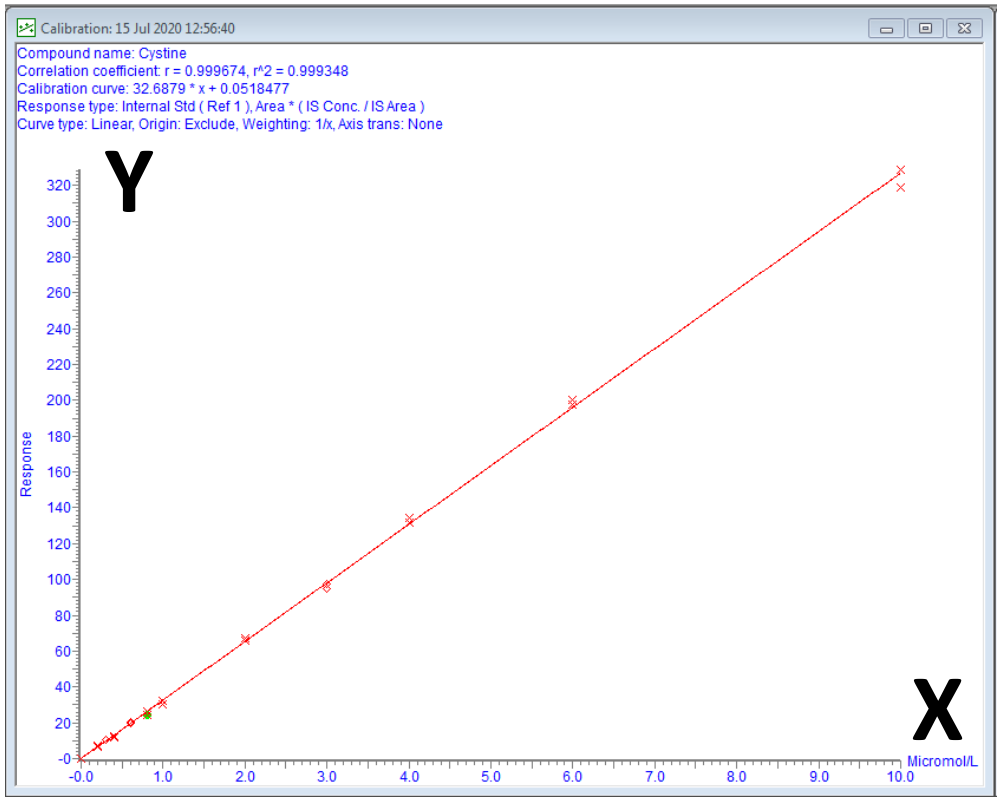

Fig. 5. Chromatogram of cystine (0.2 µM) and $d_8$ cystine obtained using an aqueous standard calibration solution.

# (3) Cystine assay

## Concentration determination : use of a Calibration curve

Y = Signal intensity
X = Cystine concentration (µM)

| Cystine µM |
|---|
| 0,2 |
| 0,4 |
| 0,8 |
| 1 |
| 2 |
| 4 |
| 6 |
| 10 |

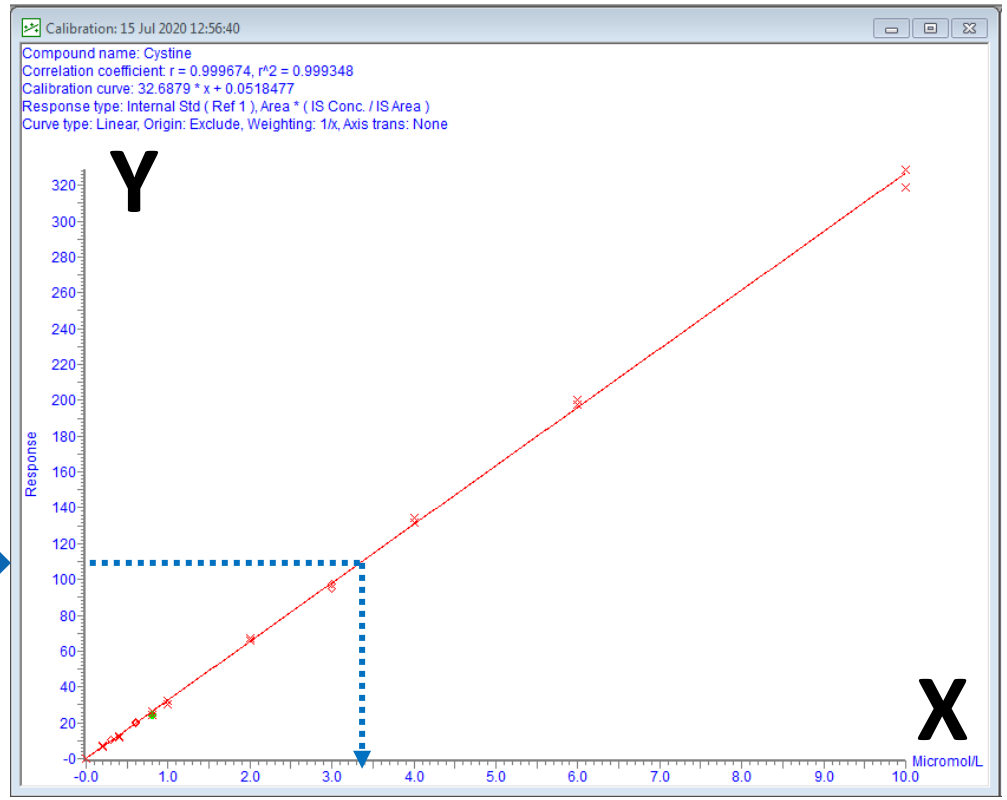


# (3) Cystine assay

## Concentration determination : use of a Calibration curve

Y = Signal intensity
X = Cystine concentration (µM)

Patient sample



# 4. Results

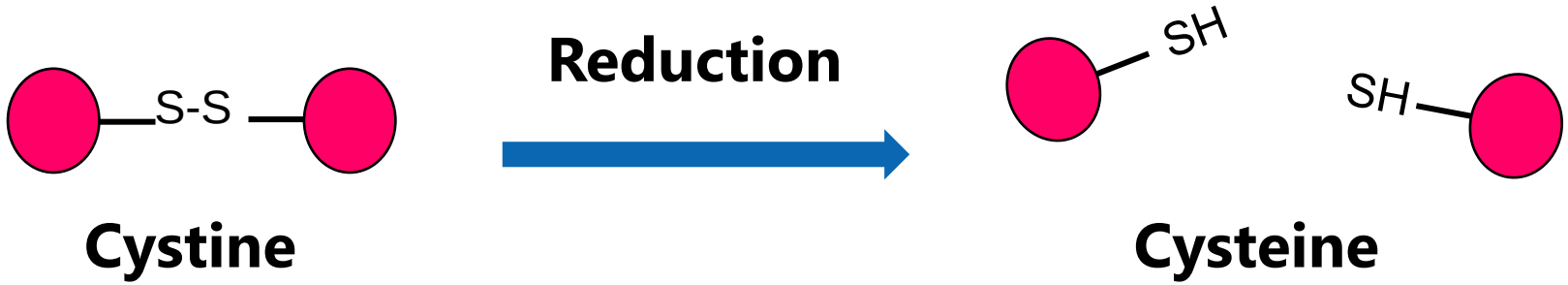
## Cystine « normalised » with protein content

- Cystine assay : nmol cystine per cell extract
- Protein determination : mg protein per cell extract
- Result expression : **nmol ½ cystine / mg protein**

# (4) Results

## Result in hemicystine ?!

- **Before** : result in nmol of **cysteine** / mg prot – Reference values




**1 molecule of CYSTINE = 2 molecules of CYSTEINE or HEMICYSTINE**

- **Now** : direct assay of Cystine (no reduction in cysteine)

Result in nmoles cystine X 2 = nmoles of hemicystine

# (4) Results

## Reference values

- Controls – Heterozygotes – Patients at diagnosis
- Mean ± 2 standard deviation (SD)

| nmoles 1/2 cystine / mg prot | Granulocytes (CUSL) | Leucocytes | |
|---|---|---|---|
| Normal values | **≤ 0,4** | **< 0,1** | |
| Heterozygotes | **< 1,2** | **< 0,7** | |
| Patients (diagnosis) | **> 2** | **> 1,5** | |

# (4) Results

## Reference values

- Controls – Heterozygotes – Patients at diagnosis
- Mean ± 2 standard deviation (SD)

| nmoles 1/2 cystine / mg prot | Granulocytes (CUSL) | Leucocytes | Gertsman 2016 / (and Nijmegen) |
|---|---|---|---|
| Normal values | **≤ 0,4** | **< 0,1** | **0,09 - 0,35** |
| Heterozygotes | **< 1,2** | **< 0,7** | **0,33 - 1,35** |
| Patients (diagnosis) | **> 2** | **> 1,5** | **0,65 - 6,05** |

# Conclusion

Reference values – Consensus ?

Sources of differences in cystine levels in different labs :

- Different cell isolation methods in the labs
  - **Mixed leucocytes**
  - **Different dextran methods**
  - **Magnetic particles…**

- Cystine assay method

Project of comparison method between BE and NL