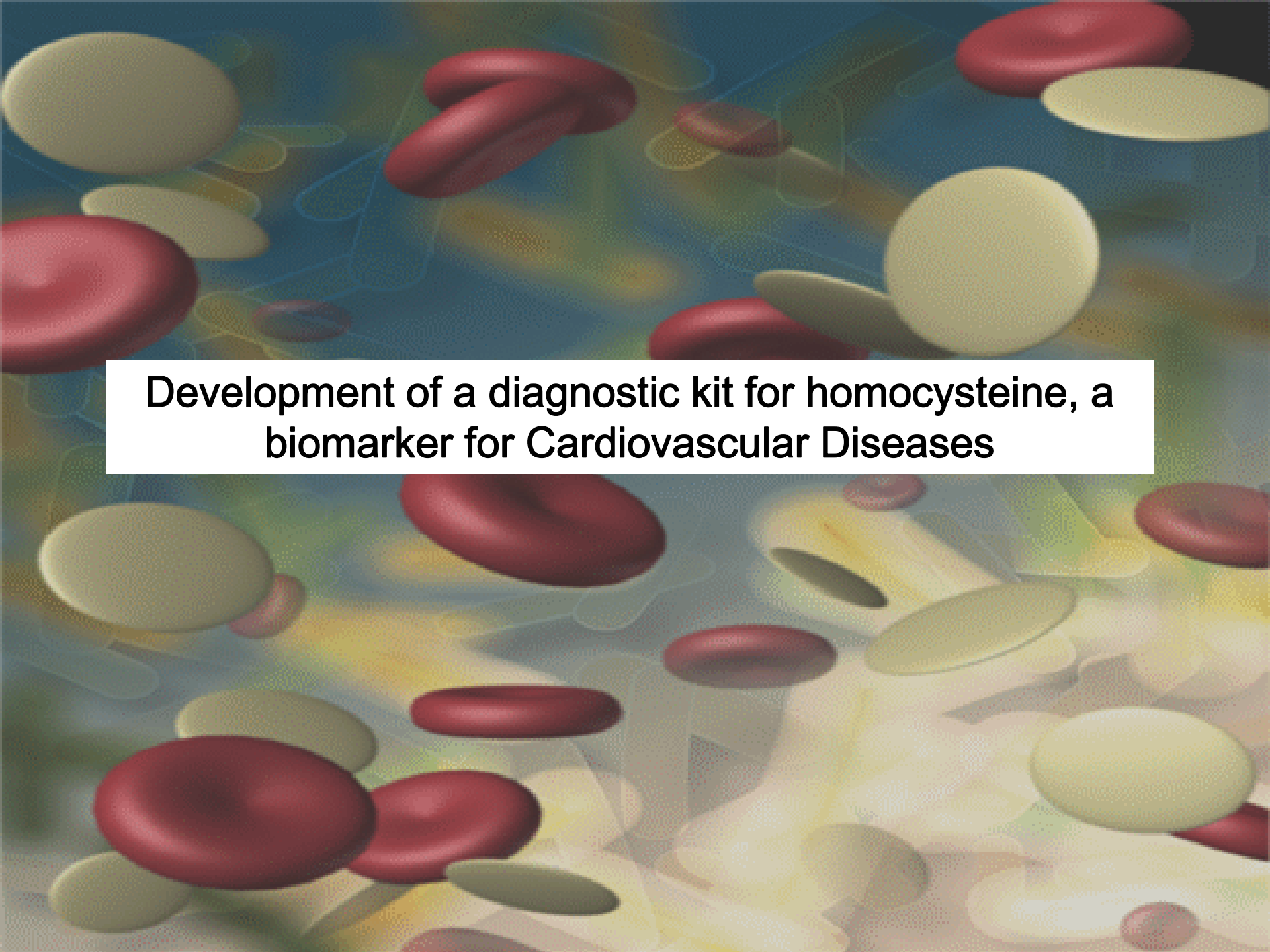
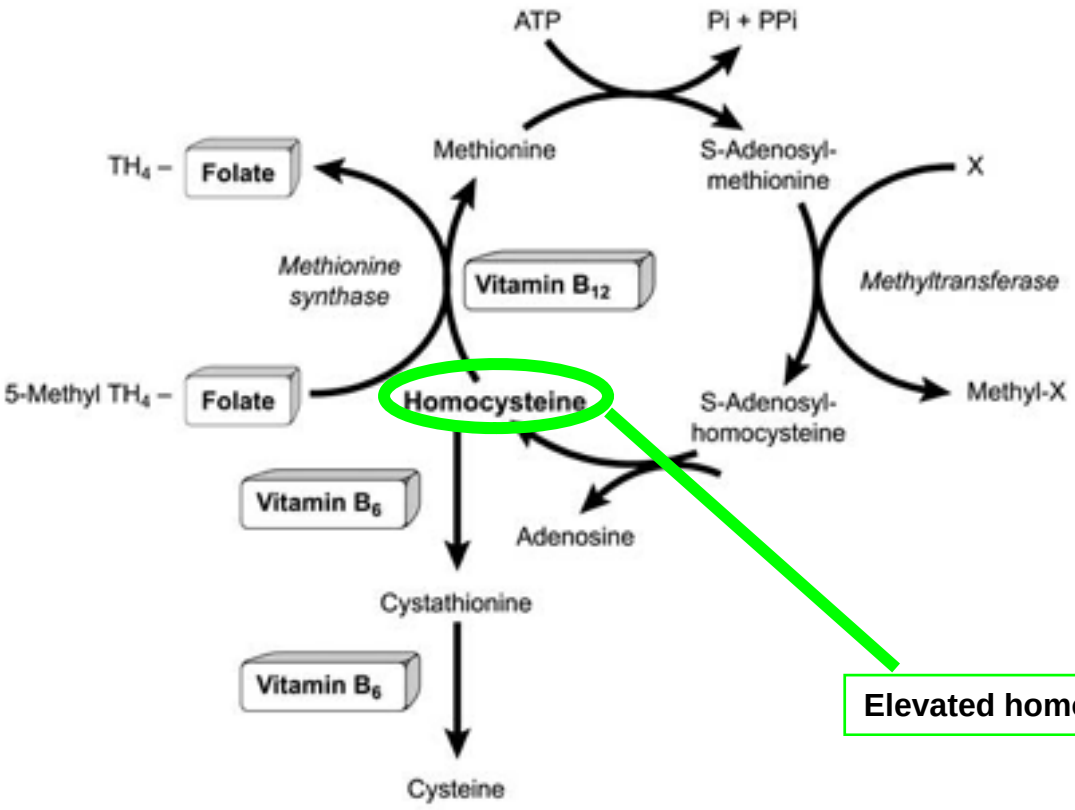


Development of a diagnostic kit for homocysteine, a biomarker for Cardiovascular Diseases

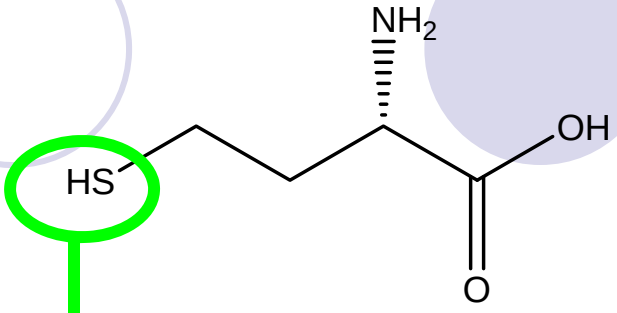
What is homocysteine?



- **Homocysteine is a sulfur-containing amino acid**
- **Produced following the methylation of target proteins**
- **Homocysteine is metabolized in the human liver.**
  - **Methionine salvage pathway**
  - **Cysteine production**

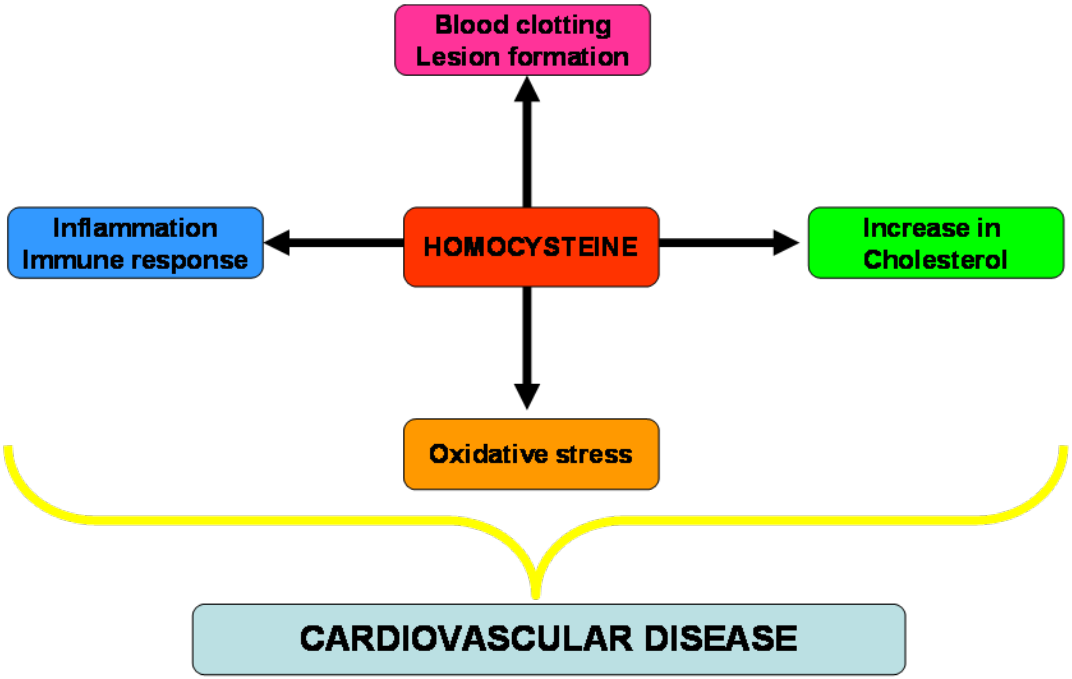
Elevated homocysteine levels can be quite toxic!

Why is homocysteine so toxic?

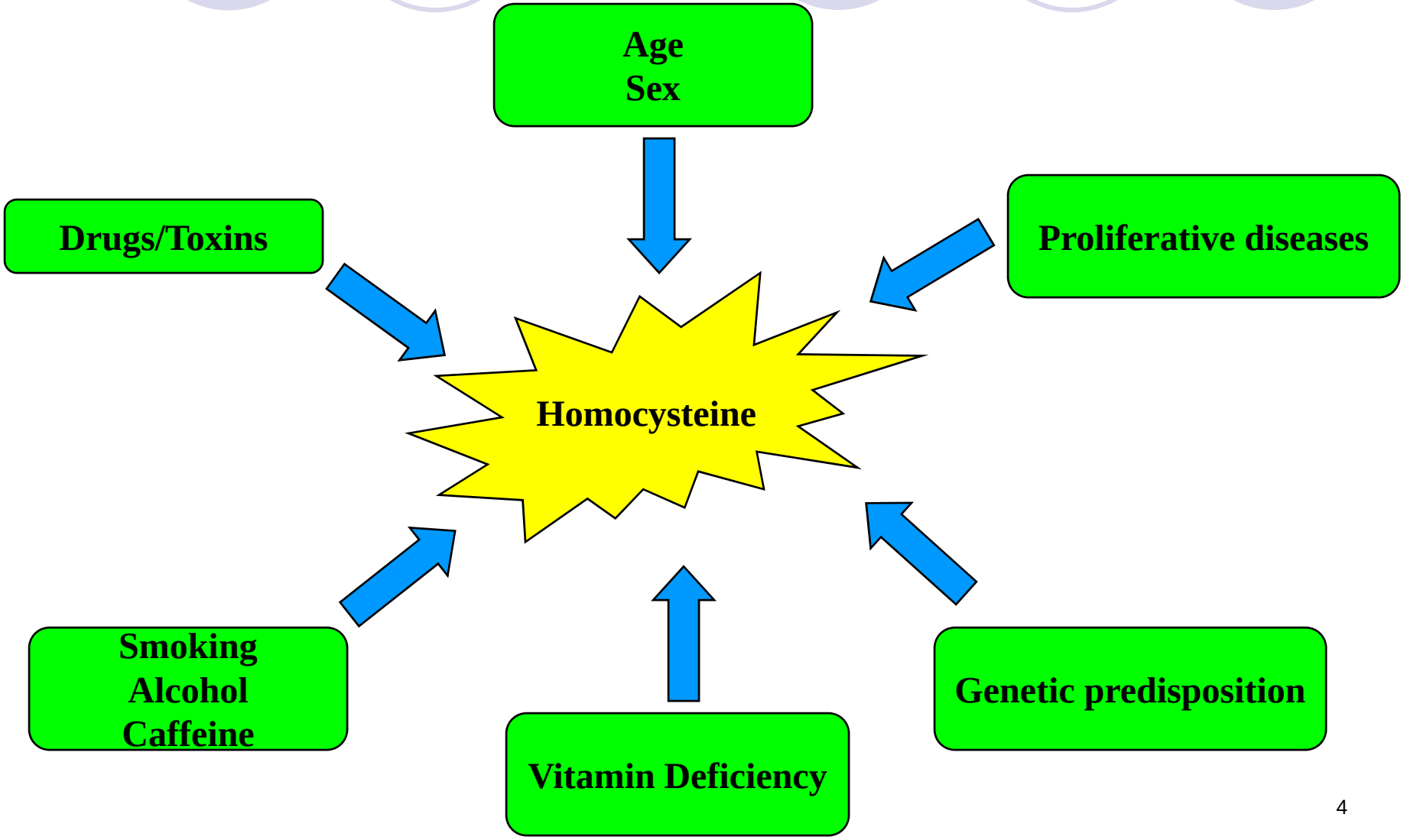


homocysteine

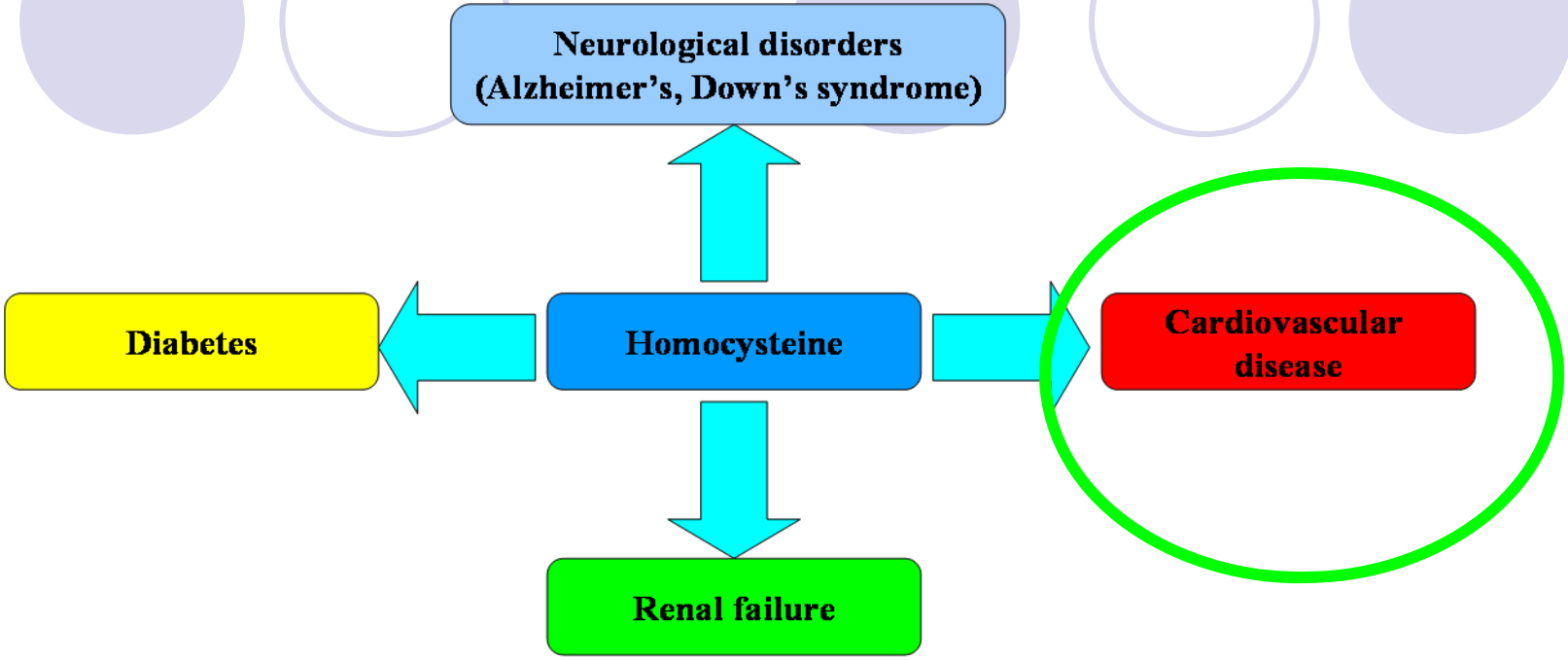
• **Highly reactive thiol (low redox potential) capable of covalently modifying various proteins and compounds involved in numerous biological processes.**

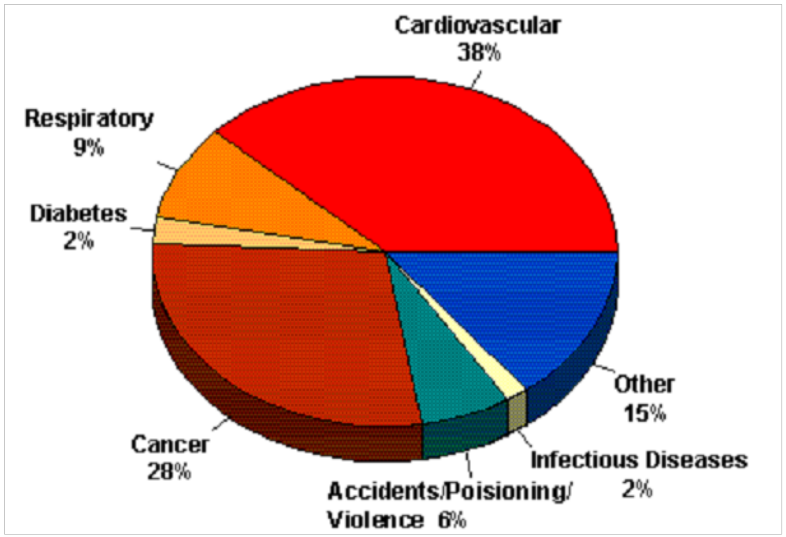


Causes of elevated homocysteine levels



Diseases associated with homocysteine

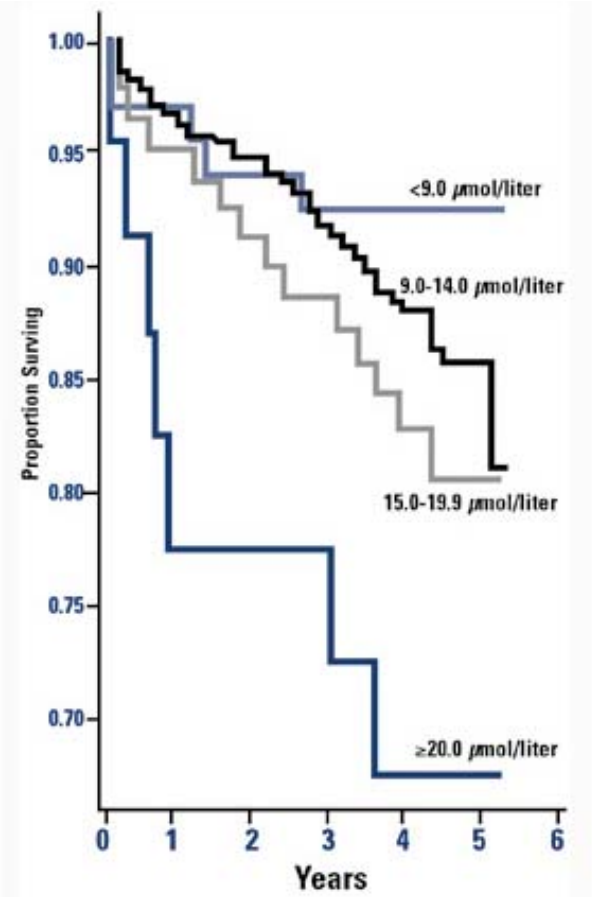




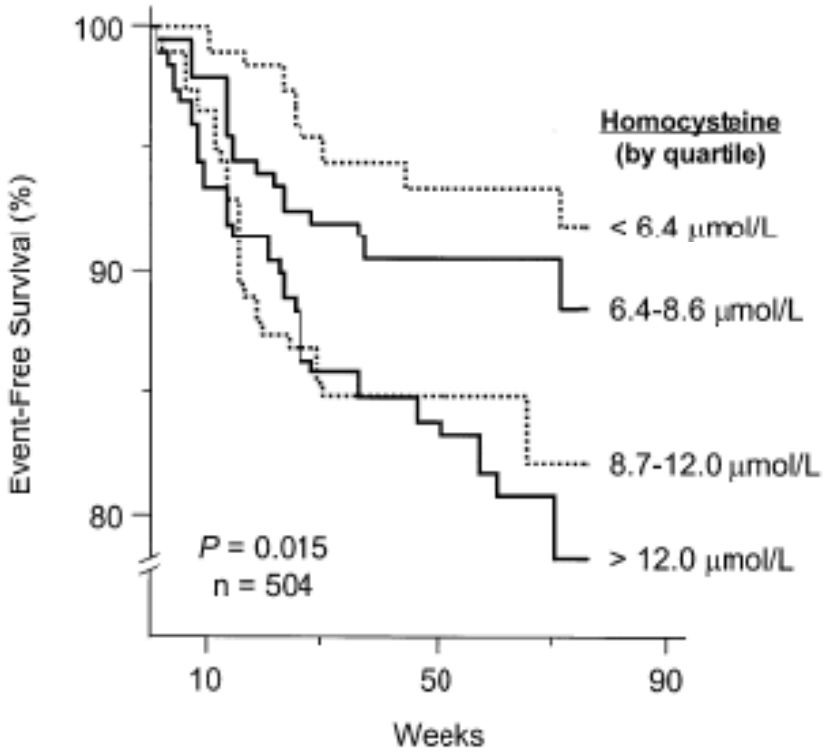
Disease distribution in Canada

Homocysteine and cardiovascular disease

A positive correlation has been established between elevated homocysteine levels and Cardiovascular disease



Nygard *et al*, N. Eng. J. 337: 230-6, 1997



Snyder *et al*, J. Am. Coll. Cardiol. 20: 40(10), 1769-76

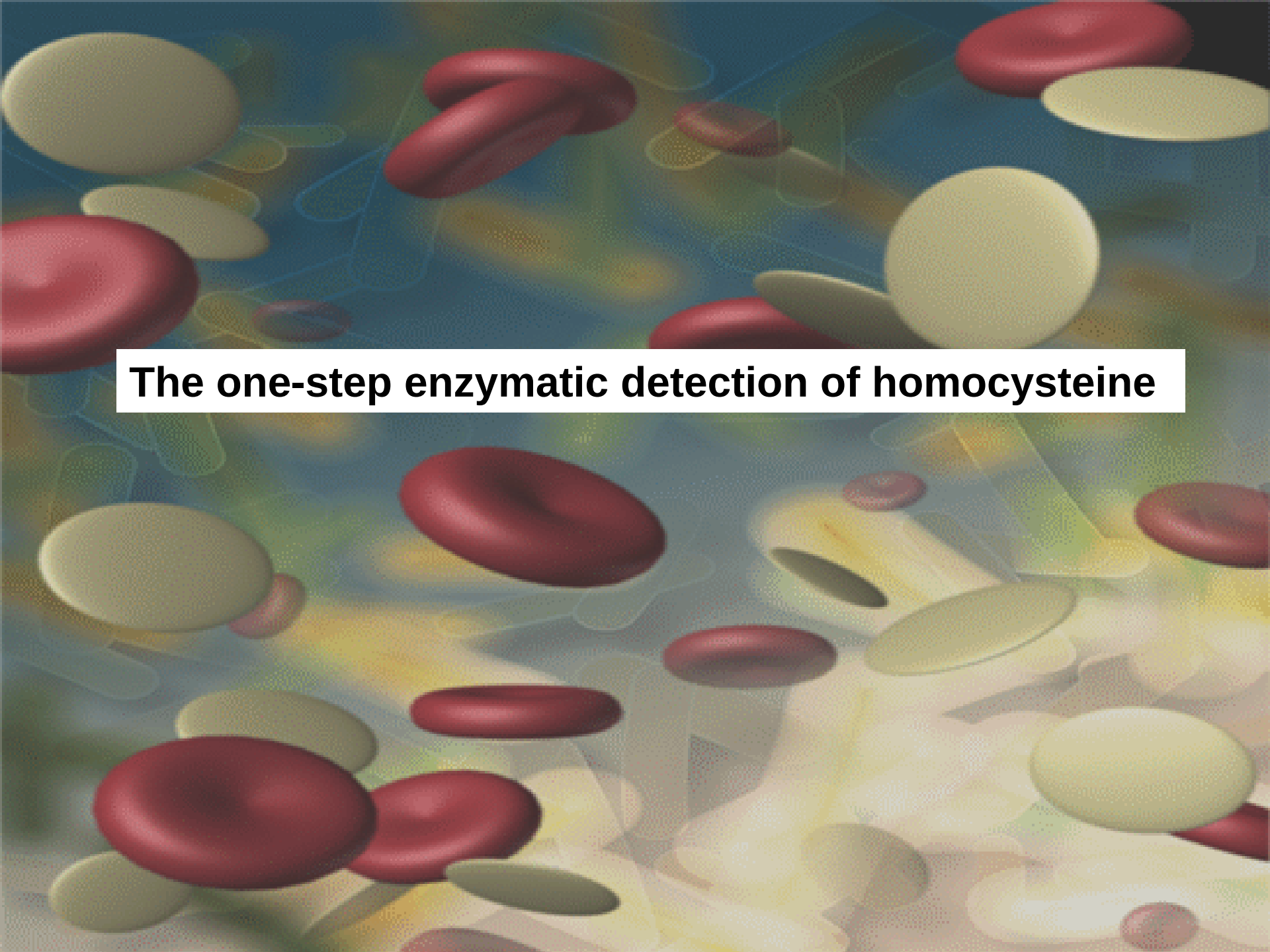
Methods for measuring CVD biomarkers

Biomarker	Drawback
Homocysteine	**Cost**
Lipoproteins (cholesterol)	**Differentiation between good cholesterol and bad cholesterol**
c-reactive protein	**Indicator of infection and inflammation**
Ascorbic acid	**Fluctuates according to diet**
Lactate dehydrogenase	**Circulating lactate dehydrogenase is also an indicator of liver failure, kidney disease, and heart failure**

Current assays for measuring the blood levels of homocysteine

	Assay	Cost	Drawback
	HPLC	65$ per assay	Sophisticated equipment Highly trained staff Expensive Not high throughput
	Immunoassays	$600 per kit	Reagents are expensive Highly trained staff Sophisticated equipment
Enzymatic assays →	Inosine dehydrogenase	FDA approved	4 enzyme steps Requires a number of cofactors
Enzymatic assays →	Lactate dehydrogenase	FDA approved	4 enzyme steps Requires a number of cofactors

Although homocysteine detection can be used as a biomarker for CVD, a cost effective and accurate method of deciphering homocysteine levels in the blood is still required.


The one-step enzymatic detection of homocysteine

The isolation of the homocysteine-metabolizing enzyme

Microbial consortium growing in a nutrient medium

Isolation of microbes

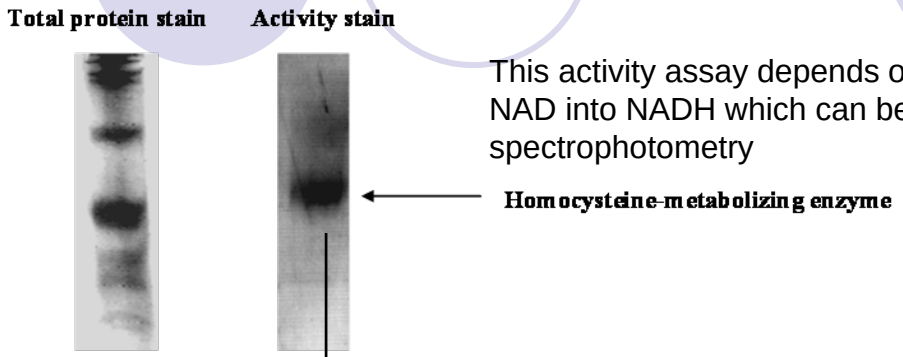
Isolation of the enzyme in 4 hours

Preparation of the enzyme for homocysteine assays

Detection of homocysteine in one-step

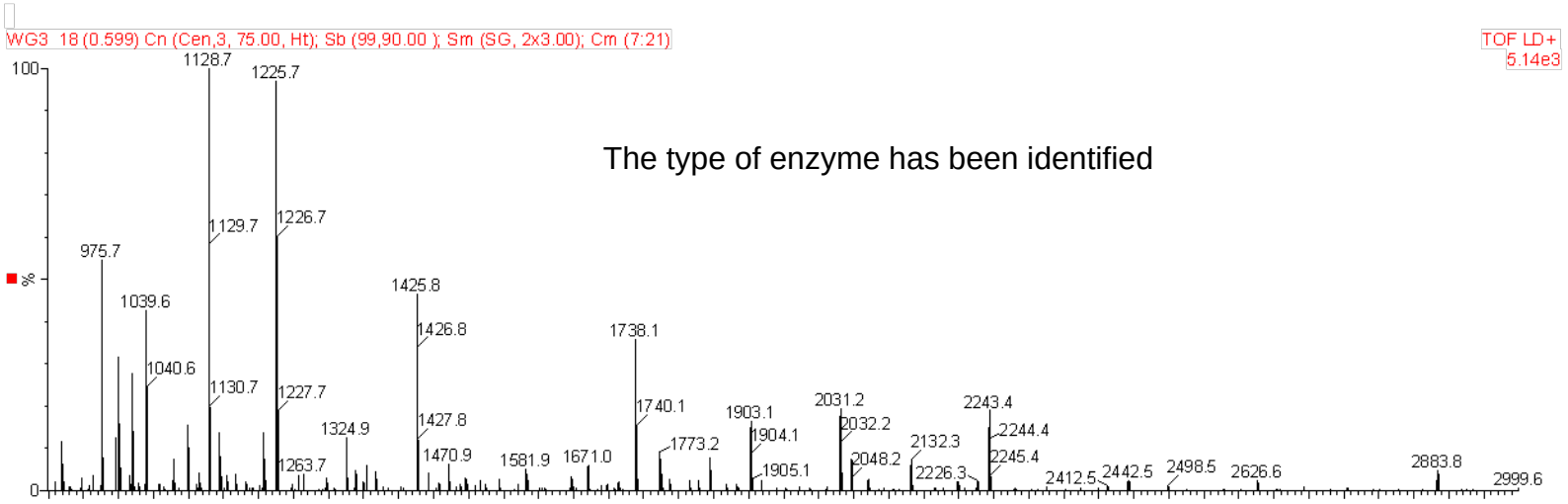
- Amount of protein isolated from one 200mL microbial culture is 30mg/mL which can run up to 150 assays
- Only a crude protein fraction is required for the accurate measurement of homocysteine.

Enzyme identification



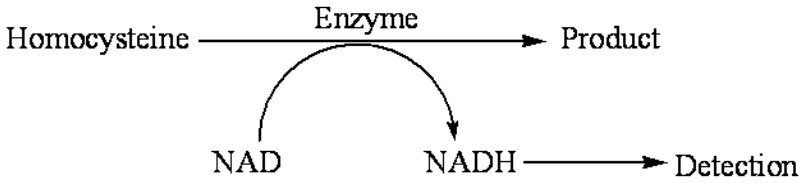
This activity assay depends on the conversion of NAD into NADH which can be detected easily by spectrophotometry

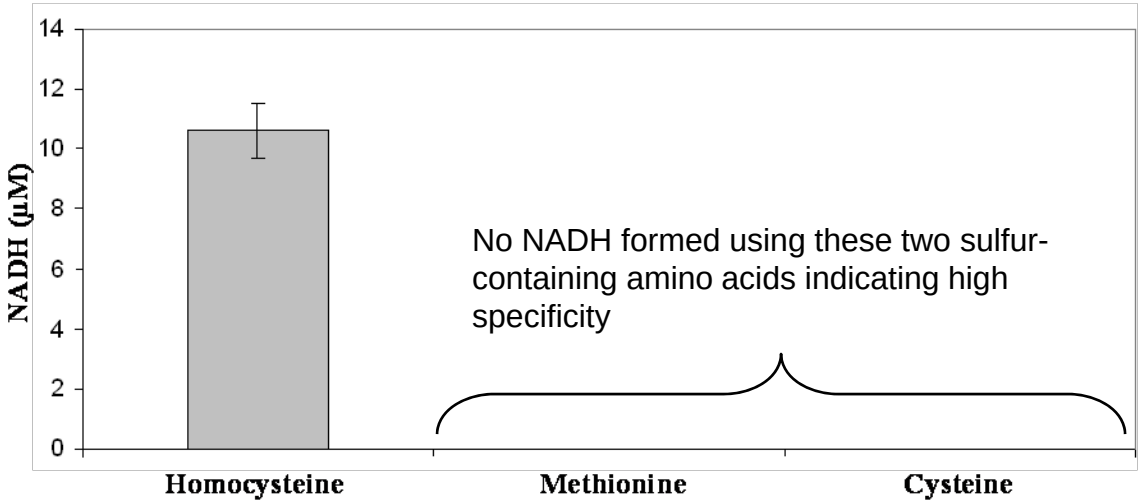
MALDI-TOF



The type of enzyme has been identified

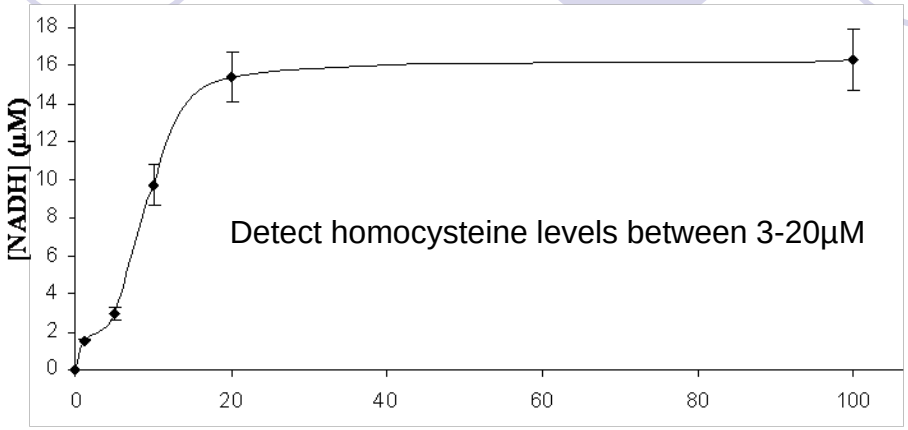
Detection of homocysteine levels by spectrophotometry

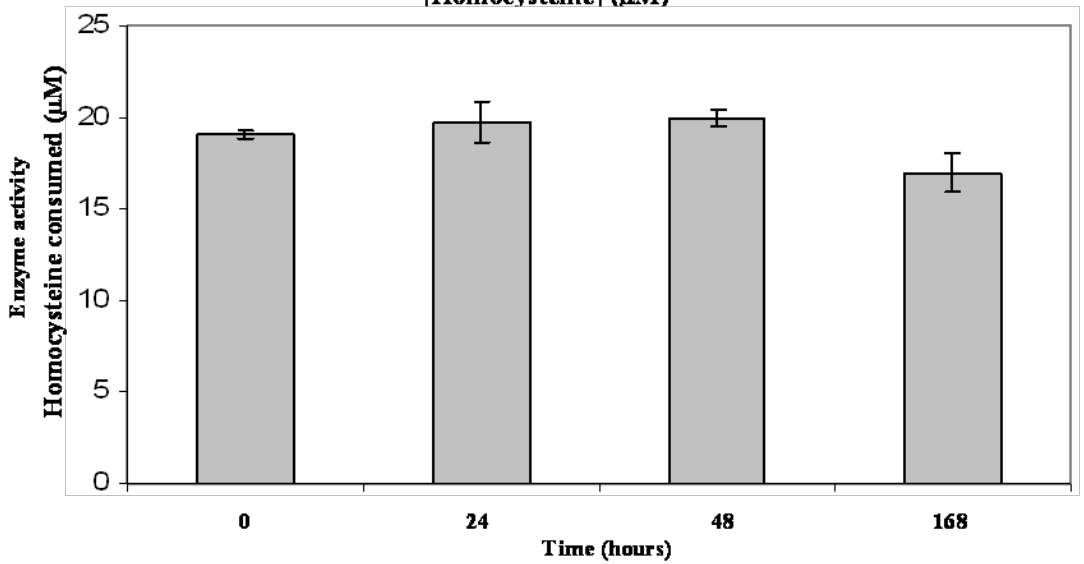




The enzymatic detection of homocysteine in this one-step method is performed by monitoring the formation of NADH, a by-product of homocysteine metabolism, with a spectrophotometer. This instrument is very cheap and found almost every clinical setting.

Enzyme sensitivity and stability

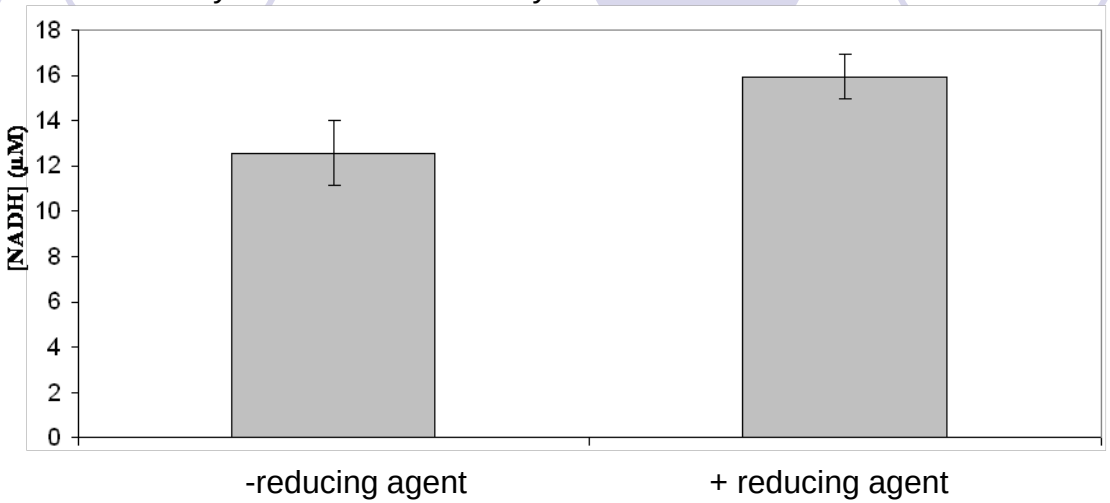




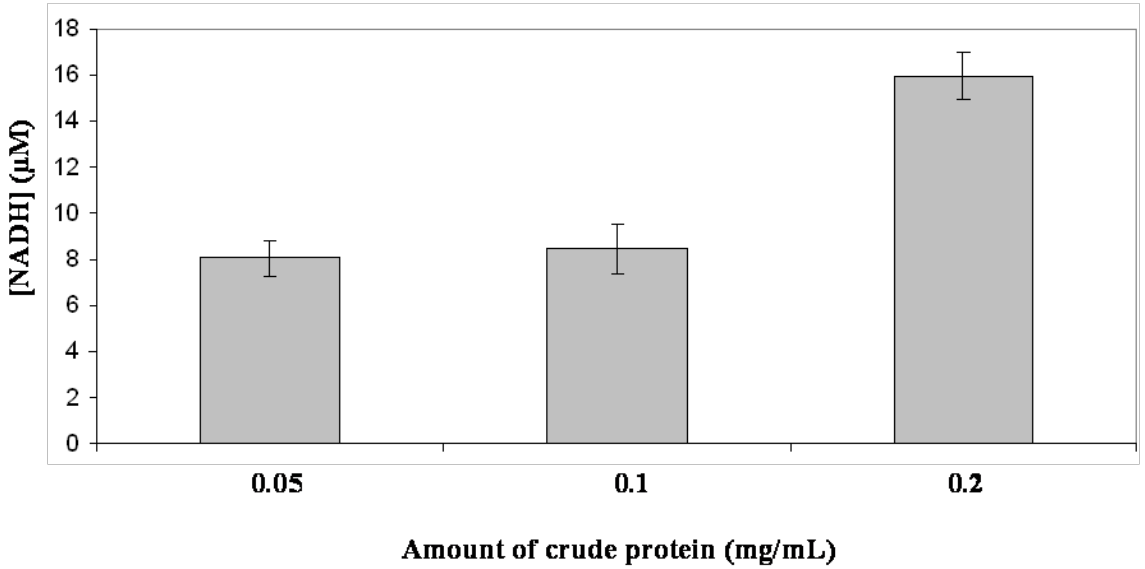
The powdered form of the enzyme is stable for 1 week

Assay optimization

Reducing agents increased accuracy of the detection assay

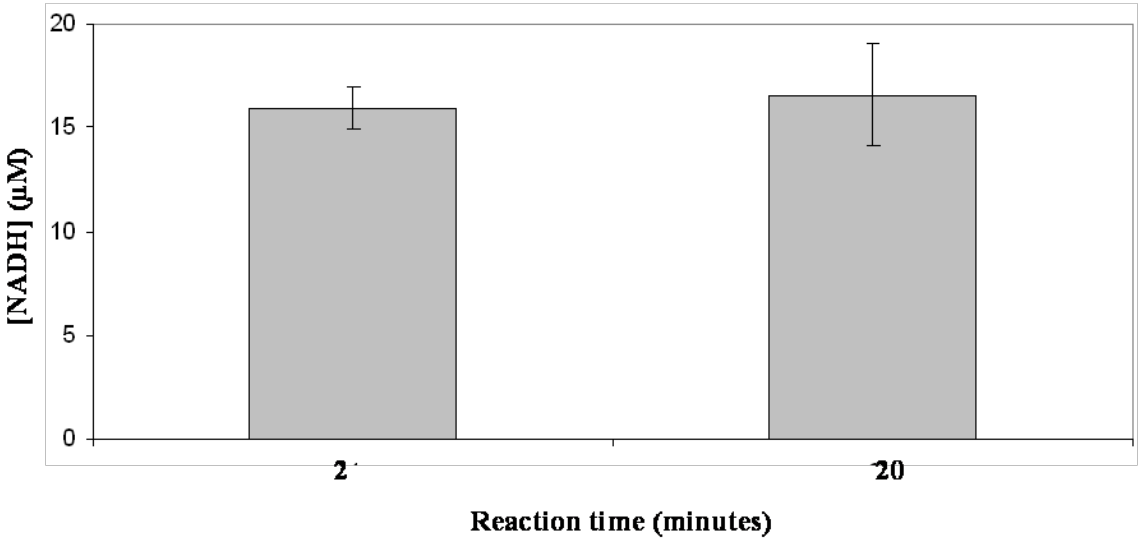


Only 0.2mg/mL of crude protein extract is required



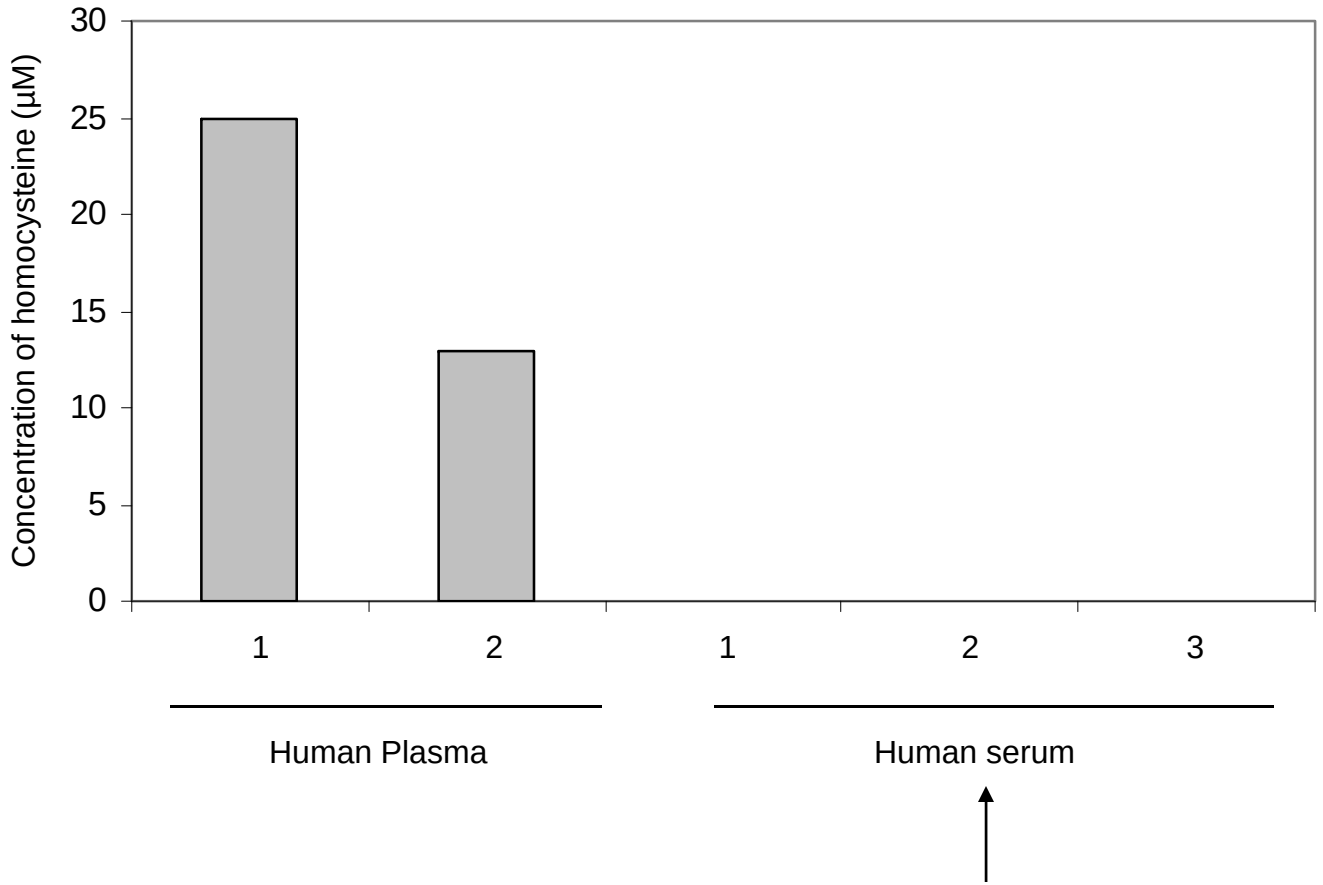
Assay optimization

Reactions can be performed for up to 20 minutes

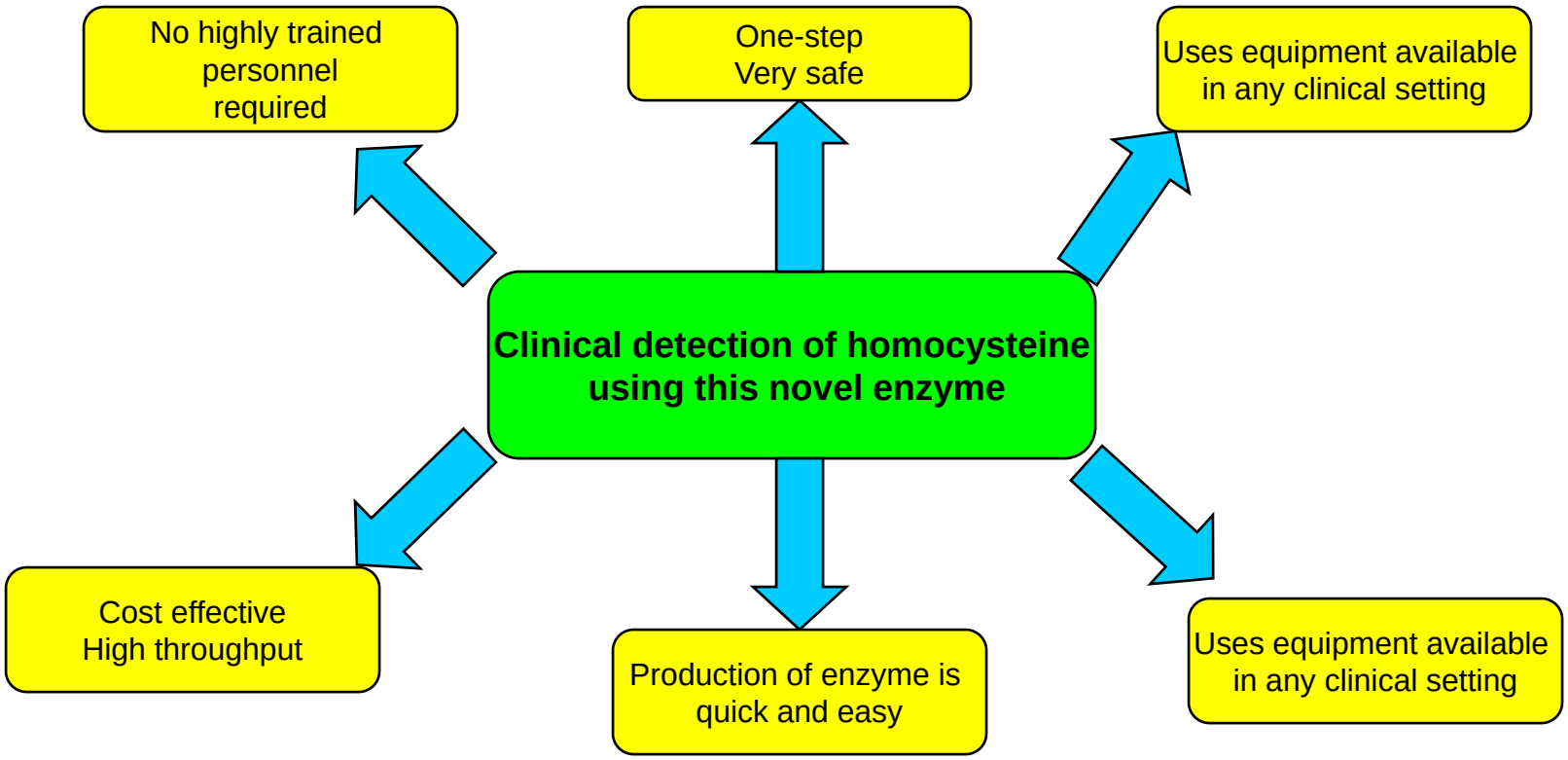


Detection of homocysteine in human blood

Human plasma samples are required for the assay.



Use of this enzyme in the clinical detection of homocysteine



Concluding remarks

- Thus, this novel homocysteine-metabolizing enzyme can provide an opportunity to develop a cost effective and efficient method for detecting this pathological biomarker

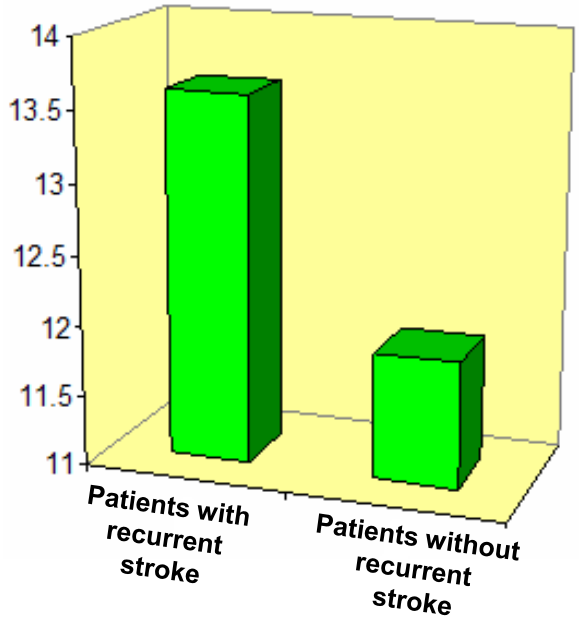
Research Team

Dr. Vasu Appanna
Project leader

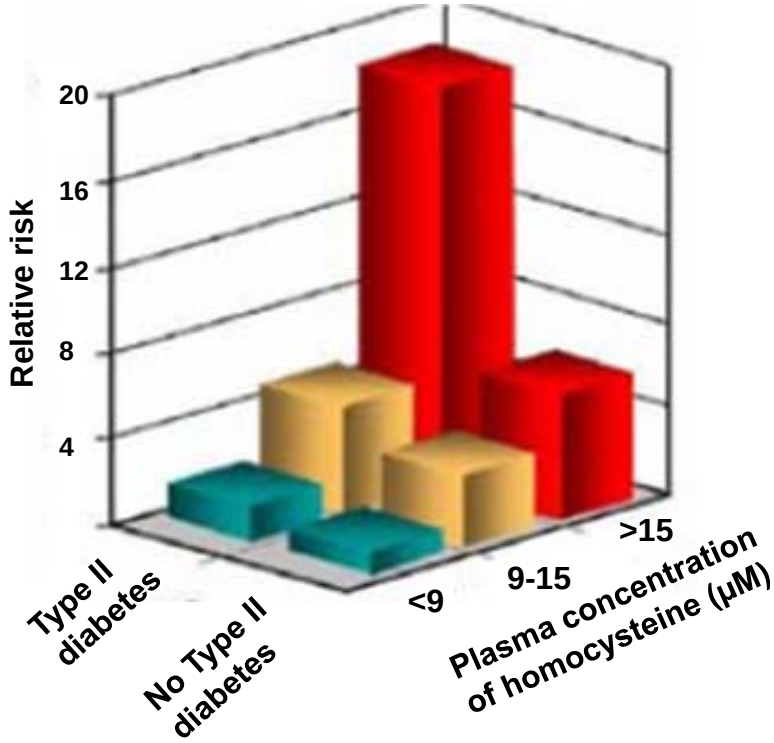
Dr. Ryan Mailloux
Research associate

Homocysteine as a predictor of stroke and diabetes

Homocysteine as a predictor of ischaemic stroke recurrence

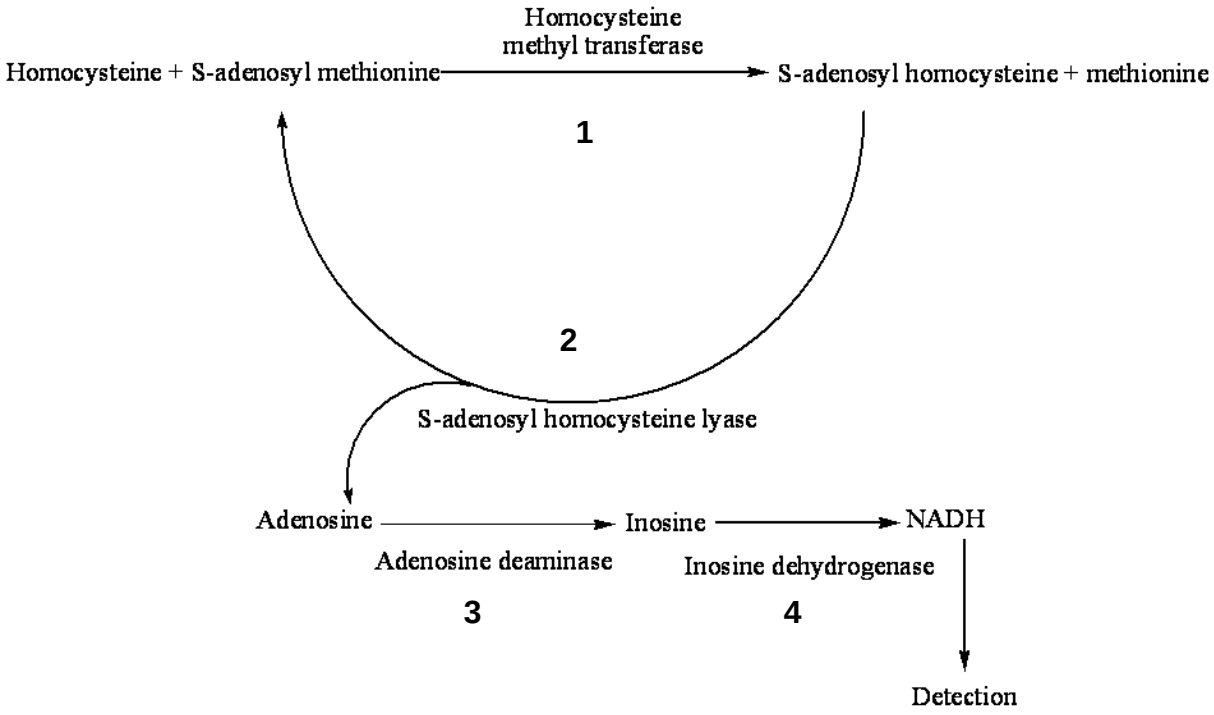


Homocysteine and diabetes promote cardiovascular disease



Enzyme detection of homocysteine

Inosine dehydrogenase method (Developed by Diazyme Laboratories



4 enzymatic steps!