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# Enzyme Analysis

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# Enzymatic Analysis

## —— Enzymatic Endpoint Assay

# Enzymatic Analysis Methods

Kinetic assay

Enzymatic Endpoint assay

- Single enzymatic quantitative analysis
- Coupled enzymatic quantitative analysis

Enzyme-linked immunosorbent assay (ELISA)

- **Single enzymatic quantitative analysis**
  - To determine the substrate
  - To determine the coenzymes
- **Coupled enzymatic quantitative analysis**
  - Dehydrogenase as the indicator enzyme
  - Other enzymes as the indicator enzyme

# I. Single enzyme quantitative analysis

## 1. Determination of the substrate

**The decrement of substrates**

The substrate can be completely convert to product, and the substrate has a special character for detection

**The increment of products**

Almost all substrates can be completely convert into products which can be specially measured

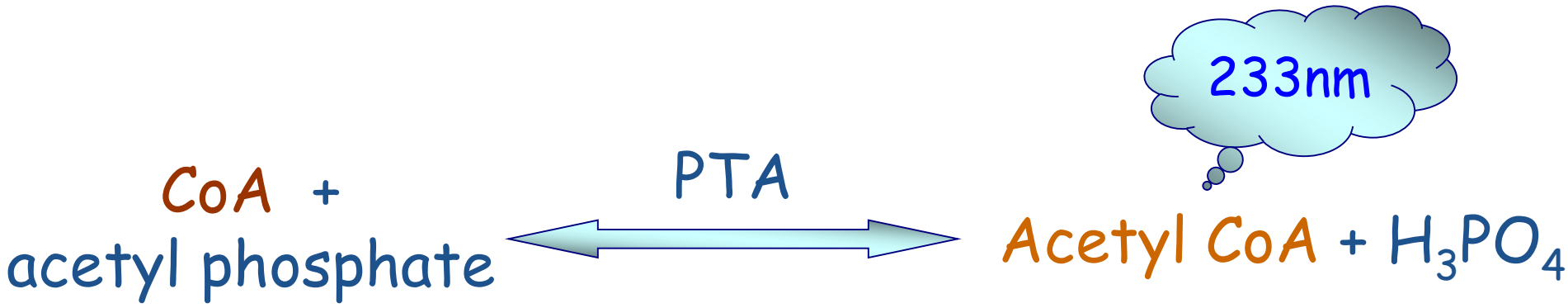
**The change of coenzyme**

Dehydrogenases utilizing NAD+ or NADP+ as coenzyme, The substrate can be measured by monitoring $A_{340}$ of NADH or NADPH

# I. Single enzyme quantitative analysis

## 2. Determination of the coenzymes

Coenzyme can be measured by single enzymatic reaction



# II. Coupled enzymatic reaction assay

When the substrate or the product can't be measured directly, the quantitative assay can be carried out by coupled another enzymatic reaction.

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C$$

Measuring the amount of C using $E_2$ as an indicator enzyme.

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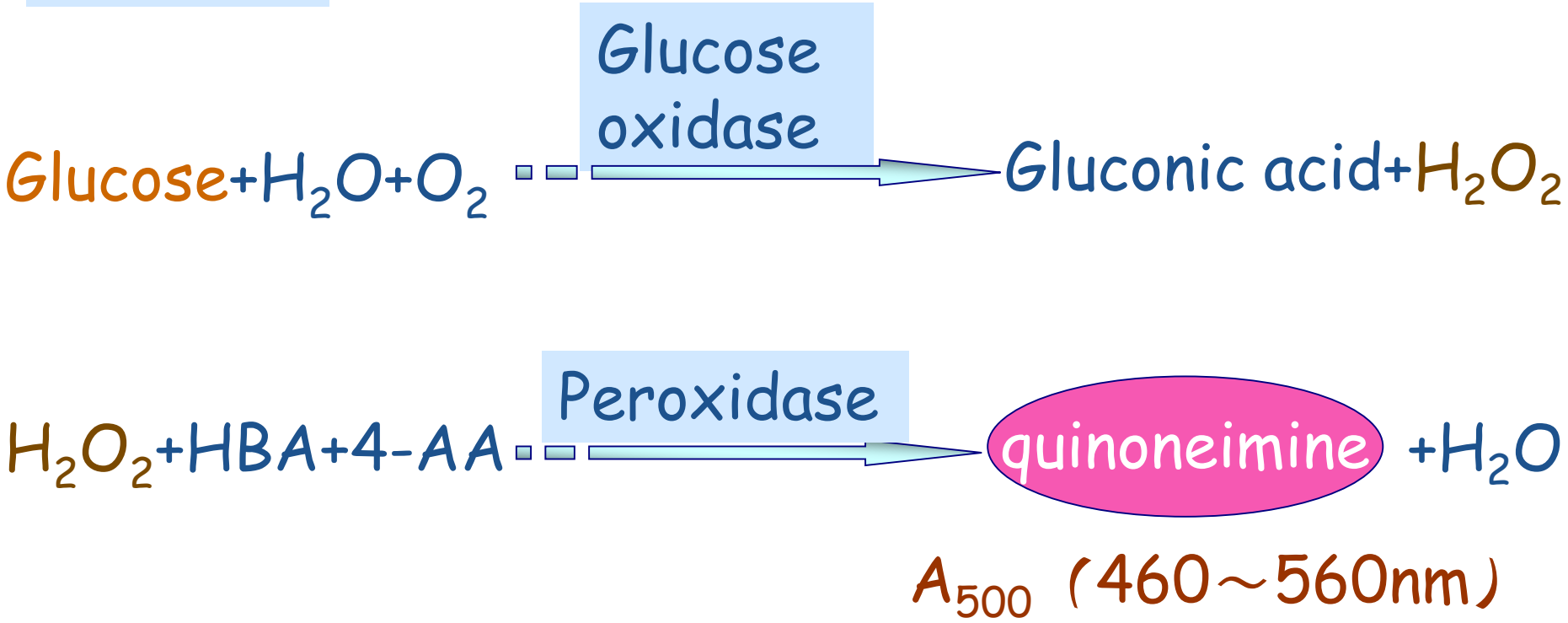
# I. Determination of blood Glucose

## (GOD-PAP Enzymatic Endpoint Method)

### Principle



# I. Determination of blood Glucose

## Reagents:

- Reaction reagent: PBS buffer, potassium ferrocyanide, EDTA-$Na_2$ peroxidase (POD), Glucose oxidase (GOD), 4-AA,HBA
- Distilled water ($dH_2O$)
- Standard glucose solution (5.55 mmol/L or 100 mg/dL)
- Serum or plasma

# Procedure

| (μL) | Blank | Standard | Test |
|---|---|---|---|
| Distilled $H_2O$ | 5 | - | - |
| Standard solution | - | 5 | - |
| Sample solution | - | - | 5 |
| Reaction reagent | 200 | 200 | 200 |

**Mix the solution in a 96 well microplate respectively, incubate for 10 min at 37℃, the blank is adjusted to zero, $A_{500}$ is read out using microplate reader.**

# I. Determination of blood Glucose

## Calculation

$$C_{Glu}\ (mmol/L) = \frac{A_{test}}{A_{Standard}} \times Cs$$

Glu. in serum or plasma:

3.89-5.83 mmol/L (70-105 mg/dL)

# Clinical significance

## Hyperglycemia

Diabetes mellitus

## Hypoglycemia

# II. Determination of Total Cholesterol (TC) in Serum

## (CHO-PAP Enzymatic Endpoint Method)

Chol ester $+H_2O$ —(Chol esterase)→ Chol+Fatty acid

Chol $+O_2$ —(Chol oxidase)→ Cholestene-3-one + $H_2O_2$

$H_2O_2$+HBA+4-AA —(Peroxidase)→ quinoneimine $+H_2O$

$A_{500}$ (460～560nm)

# Reagents

- Reaction Reagents：

  PBS, peroxidase(POD), cholesteryl esterase(COE), Cholesterol oxidase(COD), 4-AA, HBA

- Distilled water (di$H_2O$)
- Standard cholesterol solution (200 mg/dL)
- Serum or plasma (fasting overnight)

# Procedure

| (μL) | Blank | Standard | Test |
|---|---|---|---|
| Distilled $H_2O$ | 5 | - | - |
| Standard solution | - | 5 | - |
| Sample solution | - | - | 5 |
| Reaction reagent | 200 | 200 | 200 |

**Mix the solution in a 96 well microplate respectively, incubate for 10 min at 37℃, the blank is adjusted to zero, $A_{500}$ is read out using microplate reader.**

# Calculation

$$\text{Conc. of Chol. in serum} = \frac{A_{test}}{A_{standard}} \times \text{Conc. of Standard Chol.}$$

# Clinical significance

- Lipid profile is a measure of the lipid contents of the blood. It includes measurement of TG and cholesterol (TC, HDL-C, LDL-C, VLDL chol).
- Chol and TG are insoluble in water and are transported in the blood in lipoproteins, complexes of lipids with apolipoproteins.
- There are four major classes of lipoprotein: chylomicrons, VLDL, LDL and HDL. It assumes major significance in diagnosis and management of cases of dyslipoproteinemia.

# Clinical significance

## hypercholestrolemia

Genetic dyslipoproteinemia, Secondary dyslipoproteinemia, nephrotic syndrome, obesity, alcoholism, hypothyroidism, Diabetes mellitus and certain drugs， Premature atherosclerosis

## Screening for CHD

# Clinical Significance

- **Lipid and Atherosclerosis** or **CHD (Coronary Heart Disease)**

Elevated cholesterol (LDL-C) levels, there is an increased incidence of atherosclerosis and its complication

HDL-C is considered as the 'good cholesterol'

Experts agree on TC/ HDL-C ratio as a better assessment of CHD risk.

Increased TG concentrations to be positively correlated with increased risk for CHD, however, whether TG concentration represents an independent risk factor is not clear.

# Note

- This experimental result is for your **operation exam**.
- The time of final examination

**June 13-16**

- Please **submit your report (textbook)** after exam.