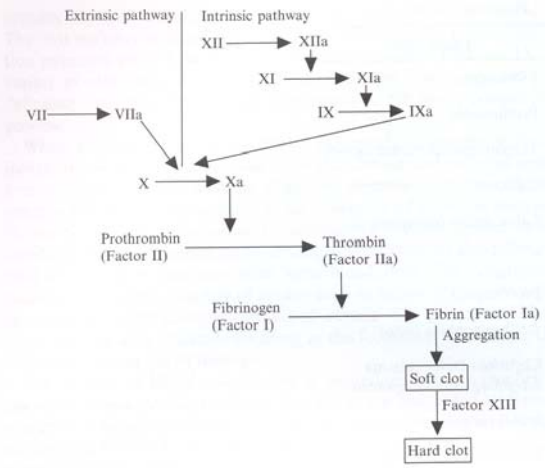
Enzymes for clinical purpose

Enzyme Engineering

Enzymes for clinical purpose

Enzyme	Therapeutic application
Ancrod (serine protease)	Anticoagulant
Tissue plasminogen activator	Thrombolytic agent
Urokinase	Thrombolytic agent
(Activated) Factors IIV and IX	Treatment of clotting disorders
Asparaginase	Treatment of some types of cancer
DNase	Treatment of cystic fibrosis
Glucocerebrosidase	Treatment of Gaucher's disease
Trypsin Papain Collagenase	Debriding/anti-inflammatory agents
Lactase Pepsin Pancrelipase Papain	Digestive aids
Superoxide dismutase	Prevention of oxygen toxicity

Blood clotting Process



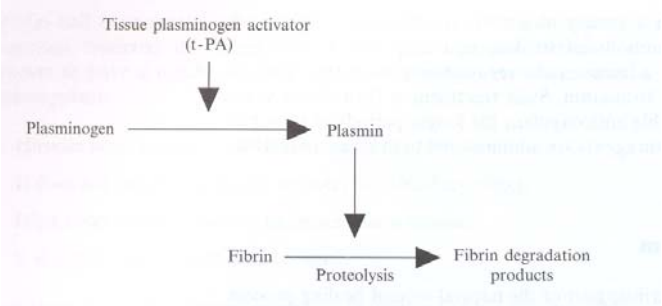
Factor number	Factor name
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Thromboplastin (tissue factor)
Factor IV	Calcium
Factor V	Labile factor (proaccelerin)
Factor VI	*
Factor VII	Proconvertin
Factor VIII	Antihaemophilic factor
Factor IX	Christmas factor (plasma thromboplastin component)
Factor X	Stuart factor
Factor XI	Plasma thromboplastin anticedent
Factor XII	Hageman factor
Factor XIII	Fibrin stabilizing factor

Blood coagulation factors

- Deficiencies of factor VIII and IX cause hereditary disease, haemophilia (cannot make blood clot)
- Vitamin K-deficiency cause similar disease
- For treating haemophilia, factor VIIa, VIII, and IX were approved as drugs. Factor VIIa and IX are enzymes

Thrombolytic Agents

- Anticoagulation agents (heparin, hirudin, or ancrod) are important for the persons with high risk of strokes and heart failure
- Blood clot within blood vessel is called thrombus
- Thrombolytic agents degrade thrombus



Thrombolytic Agents

- t-PA (tissue plasminogen activator) activates plasmin, which can degrade blood clot
- t-PA is a serine protease with 5 domains
- Engineered t-PA with two domains are commercialized from 1987
- Urokinase, streptokinase, and staphylokinase have the thrombolytic activity
- Urokinase is produced by kindney, while the other two enzymes are produced by bacteria
- Bacterial kinases do not have proteolytic activities, but indirectly activates plasmin

Asparaginase

- Asparagine can be synthesized by normal tissue, but not by some malignant (cancer) cells
- Rapid depletion of asparagine from the blood can kill a certain type of leukemia cells, but have limited effects on normal cells
- PEGylated asparaginase from *E. coli* or *Erwinia* are tested for clinical trials

DNase for treating cystic fibrosis

- Cystic fibrosis is a genetic disease, affecting 1 in 25000 newborns
- The gene of chloride channel is mutated
- The mucus of the patient is extremely viscous
- The problem of the channel protein increases the rate of bacterial infection in the lung, triggers immune response, and attract large numbers of neucrophils → Accumulation of chromosomal DNA
- DNase I is spayed in the lung

Other enzymes

- Removing foreign matter or dead tissue allows rapid healing
- Chymopapain, a proteolytic enzyme from papaya tree, is used to treat disk
- Chymotrypsin or bromelaines (a plant protease) repress the inflammatory system by degrading cytokines
- α-amylase, lactase, pepsin, papain, etc. are being used as digestive aids

Enzymes for Diagnostics

- World market of 100 million $
- Alkaline phosphatase and peroxidase are the two enzymes having biggest market, each having 20 million $
- Most of diagnostic enzymes are produced by Roche or Genzyme
- Widely used for EIA (enzyme immunoassay systems)
- Factors that diagnostic enzyme must have
  - Specificity
  - Kinetic property (suitable K_m and k_{cat} values)
  - Cost
  - Enzyme stability

Enzyme	Source	Application
Acetyl cholinesterase	Bovine erythrocytes	Analysis of organophosphorus compounds such as pesticides
Alcohol dehydrogenase	Yeast	Determination of alcohol levels in biological fluids
Alkaline phosphatase	Calf intestine and kidney, recombinant (*Picca* sp.)	Conjugation to antibodies allows its use as an indicator in ELISA systems
Arginase	Beef liver	Determination of L-arginine levels in plasma and urine.
Ascorbate oxidase	*Cucurbita* species	Determination of ascorbic acid levels; eliminates intereference by ascorbic acid
Cholesterol esterase	Pig/beef pancreas, *Pseudomonas* sp., Recombinant (*Streptomyces* sp.)	Determination of serum cholesterol levels
Creatine kinase	Rabbit muscle, beef heart, pig heart	Diagnosis of cardiac and skeletal malfunction
Glucose-6–phosphate dehydrogenase	Yeast, *Leuconostoc mesenteroides*	Determination of glucose and ATP in conjunction with hexokinase
Glucose oxidase	*Aspergillus niger*	Determination of glucose in biological samples in conjunction with peroxidase; a marker for ELISA systems
Glutamate dehydrogenase	Beef liver	Determination of blood urea nitrogen in conjunction with urease
Glycerol kinase	*Candida mycoderma, Arthrobacter* sp.	Determination of triglyceride levels in blood in conjunction with lipase
Glycerol-3-phosphate dehydrogenase	Rabbit muscle	Determination of serum triglycerides
Hexokinase	Yeast	Determination of glucose in body fluids
Peroxidase	Horseradish	Indicator enzyme for reactions in which peroxide is produced
Phosphoenolpyruvate carboxylase	Maize leaves	Determination of CO_2 in body fluids.
Urease	Jack bean	Determination of blood urea nitrogen; marker enzyme for ELISA systems
Uricase	Porcine liver	Determination of uric acid
Xanthine oxidase	Buttermilk	Determination of xanthine and hypoxanthine in biological fluids

Assay methods

- End-point vs kinetic methods
  - Kinetic method is quicker, but K_m must be high enough to make linearity between [S] and velocity
- Two commonly used methods are dehydrogenases and oxidases
  - Dehydrogenase convert NAD^+ to NADH
  - NADH have absorbance at 340nm
  - Oxidase produces hydrogen peroxide
  - Second enzyme such as peroxidase is used for detecting H_2O_2

Glucose Assay

1

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} \boxed{\text{quinoneimine}} + 4H_2O$$

2

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose 6-phosphate} + \text{ADP}$$

$$\text{Glucose 6-phosphate} + NAD^+ \xrightarrow{\text{G6P-DH}} \text{gluconate 6-phosphate} + \boxed{\text{NADH}} + H^+$$

For long term glucose level, the level of glycosylation of hemoglobin in the blood is monitored

Enzymes for cholesterol assay

- Atherosclerosis (동맥경화) and plasma cholesterol level are correlated
- LDL is linked to Atherosclerosis, but HDL rather have protective effect

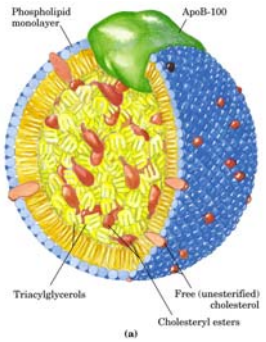


table 21–2

Major Classes of Human Plasma Lipoproteins: Some Properties

		Composition (wt %)				
Lipoprotein	Density (g/mL)	Protein	Phospholipids	Free cholesterol	Cholesteryl esters	Triacylglycerols
Chylomicrons	<1.006	2	9	1	3	85
VLDL	0.95–1.006	10	18	7	12	50
LDL	1.006–1.063	23	20	8	37	10
HDL	1.063–1.210	55	24	2	15	4

Source: Modified from Kritchevsky, D. (1986) Atherosclerosis and nutrition. *Nutr. Int.* **2,** 290 – 297.

Enzymes for cholesterol assay

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{4-cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_2O$$

- HDL cholesterol level is checked after other particles are removed by phosphotunstic acid and magnesium ions
- The level of triglyceride of the blood is also detected by the following method

Enzymes for cholesterol assay

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{glycerol 3-phosphate} + \text{ADP}$$

$$\text{Glycerol 3-phosphate} + O_2 \xrightarrow{\text{glycerol 3-phosphate oxidase}} \text{dihydroxacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{chlorophenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + HCl + 2H_2O$$

Assays for blood urea

- Elevated level of urea is indicator of kidney disease

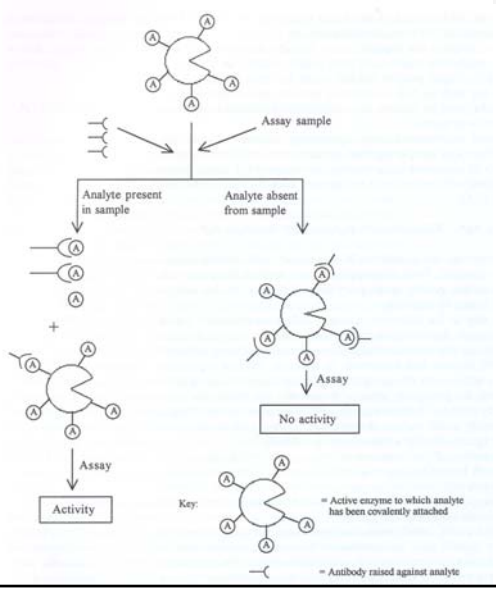
$$\text{Urea} + H_2O \xrightarrow{\text{urease}} \text{ammonia} + CO_2$$

- Ammonia react with phenol and hypochlorite in the presence of sodium nitroprusside, forming blue colored complex

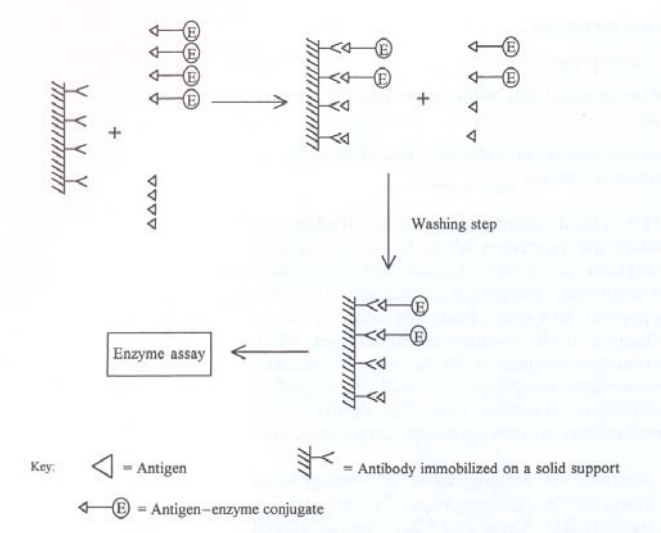
Enzyme immunoassay (EIA)

- Radioimmunoassay (RIA) has many disadvantages and is replaced by enzyme immunoassay (EIA)
- Enzymes, such as alkaline phosphatase, horseradish peroxidase, β-galactosidase, are linked to antibody and used for detection
- Horseradish peroxidase
- Alkaline phosphatse : PNPP is hydrolyzed and turned to yellow
- β-galactosidase : ONPG is used as substrate

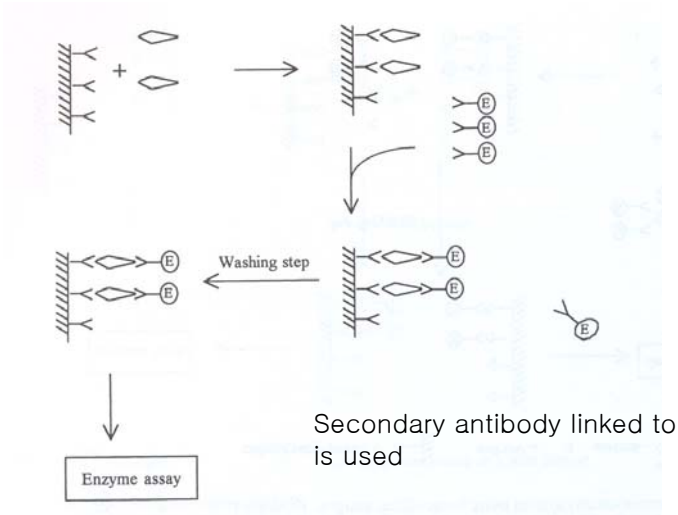
Enzyme multiplied immunoassay technique (EMIT)



Competitive solid phase EIA

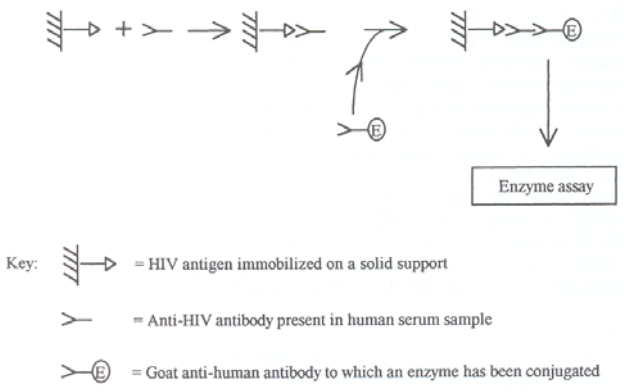


Enzyme-linked immunosorbant assay (ELISA)

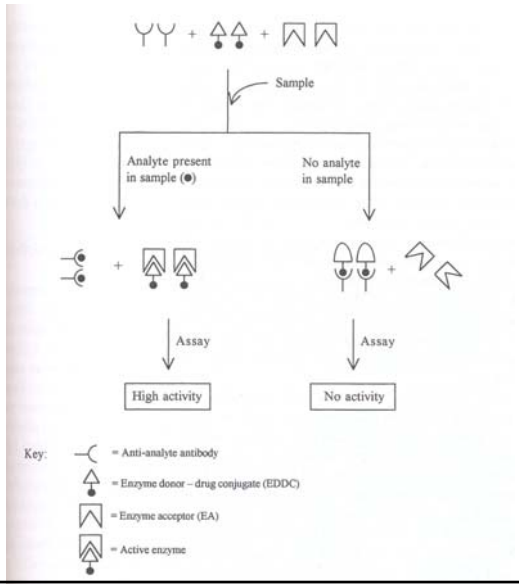


Secondary antibody linked to enzyme is used

Another type of ELISA



Cloned enzyme immunassay system (CEDIA)



Immune complex transfer EIA