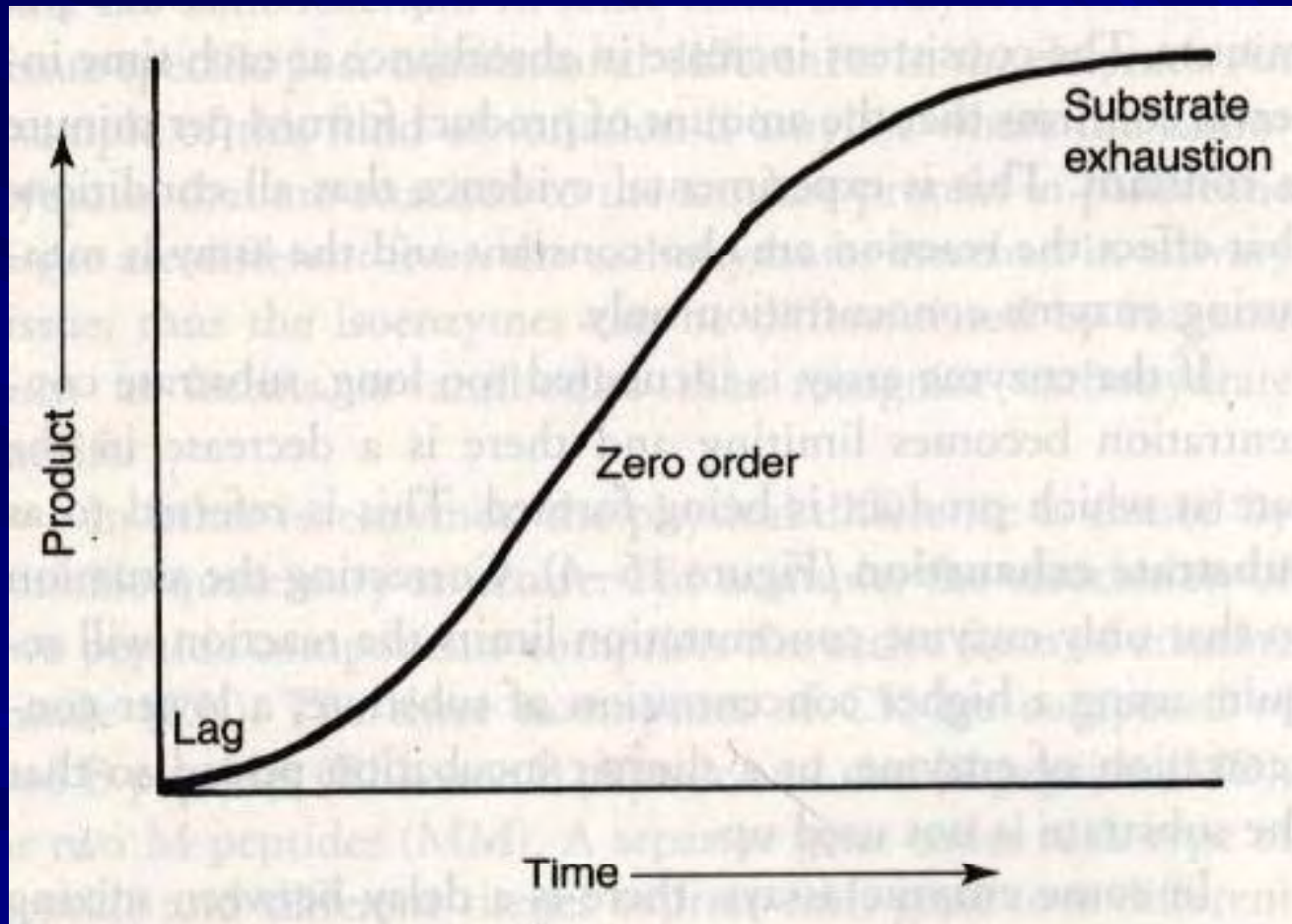


ENZYME ACTIVITY DETERMINATION OF ENZYMES AS:

- **REAGENTS**

- **ANALITES**

PROGRESS CURVE



AMINOTRANSFERASE ACTIVITY DETERMINATION



PLP



ALANINE TRANSAMINASE (ALT, GPT)

α -Ala + α -KG



PLP

Pyr + α -Glu

ASPARTATE TRANSAMINASE (AST, GOT)

α -Asp + α -KG



PLP

Oxa + α -Glu

METHODS

■ **COLORIMETRIC** (*Reitman-Frankel*)

Reaction with *2,4-Dinitrophenyl hydrazine*

METHODS

UV SPECTROPHOTOMETRIC *(Karmen)*

1) *ALT*



2) *AST*



AMINOTRANSFERASES

SPECIMEN:

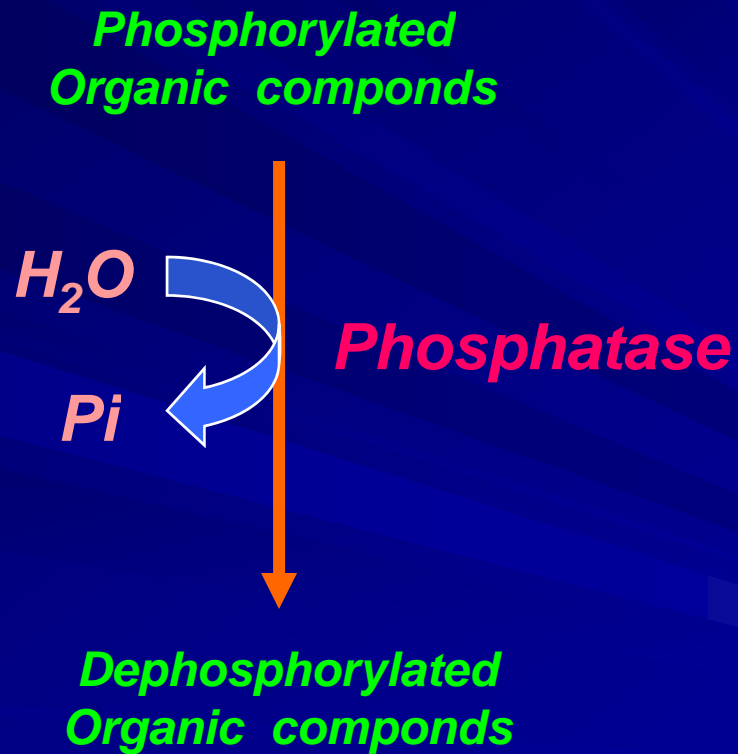
- *Serum or plasma can be used , but heparinized plasma is not suitable for ALT when TRIS buffer is used*
- *Hemolysis increases AST*
- *Serum is stable for one week at 4°C and more in -20°C*

PHOSPHATASES

Alkaline Phosphatases (ALP)

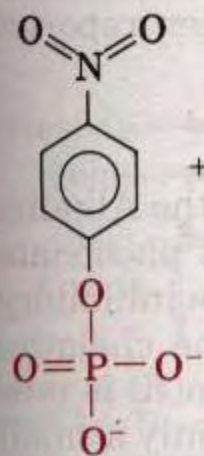
Acid Phosphatases (ACP)

BASIS OF PHOSPHATASE ACTIVITY DETERMINATION



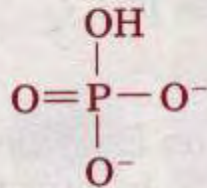
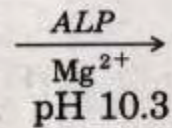
SUBSTRATES

- β -Glycerophosphate (*Kay, Bodansky*)
- Phenylphosphate (*King & Armstrong*)
- 4-Nitrophenylphosphate (4-NPP)
(*Pranitrophenylphosphate, PNPP*)
(*Bessy, Lowry, Brock*)

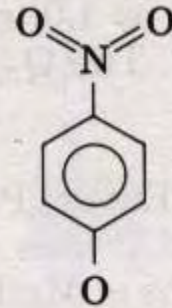


**4-Nitrophenyl
phosphate
(colorless)**

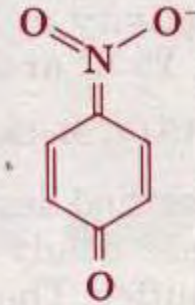
+ HOH



**4-Nitrophenoxide
(colorless benzenoid form)**



$\xrightarrow[\text{at alkaline pH}]{\text{Rearranges}}$



**4-Nitrophenoxide
(yellow, quinonoid form)**

AMINOALCOHOL BUFFERS

- **2-Amino-2-methyl-1-propanol (AMP)**
- **Diethanolamine (DEA)**
- **Ethylaminoethanol (EAE)**

ALKALINE PHOSPHATASE

METHOD:

- *Using PNPP*
- *At pH = 10.3*
- *End Point Or Kinetics*

ALKALINE PHOSPHATASE

SPECIMEN:

- *Diet*
- *Position & Stasis*
- *Serum or Heparinized Plasma*
- *Hemolysis*
- *Increasing by Storage*

LACTATE DEHYDROGENASE ACTIVITY DETERMINATION

Pyruvate + NADH + H⁺

LD



Lactate + NAD⁺

METHODS

■ COLORIMETRIC

Reaction with *2,4-Dinitrophenyl hydrazine*

■ UV SPECTROPHOTOMETRIC



SAMPLE

- Serum or Heparinized Plasma
- Activity, Stasis
- Unstability
- Hemolysis

CV% OF EQAP : AST

Kit	87-1	87-2	88-1	88-2	88-3	89-1	89-2	89-3	90-1	90-2
Pa-A	12.97	10.39	9.45	9.61	11.60	9.29	9.27	8.85	8.35	8.42
PA-M	22.11	17.11	19.74	17.47	20.76	15.97	19.08	21.27	20.55	9.51
Zi-A	-	-	-	-	-	-	-	-	-	-
Zi-M	25.49	25.15	30.15	15.04	22.72	36.33	15.60	14.76	15.80	26.02
Ma-A	-	-	9.52	8.65	-	9.74	12.12	7.18	11.35	17.65
Ma-M	-	-	-	-	-	-	-	-	-	-
EI-A	20.48	15.67	6.86	20.74	16.58	10.18	7.26	8.13	10.47	8.7
Bi-A	-	9.96	9.54	11.88	10.01	8.94	11.17	8.3	9.45	10.48
Ro-A	-	-	-	-	-	-	-	-	4.09	3.69

Average EQAP CV%	Allowable CV%	CCV%	Indian CCV%
9.8	5.0	12.5	12.5

8.35	8.42	8.85	9.27	9.29	9.45	9.61	10.39	11.60	12.97
------	------	------	------	------	------	------	-------	-------	-------

CV% OF EQAP : ALT

Kit	87-1	87-2	88-1	88-2	88-3	89-1	89-2	89-3	90-1	90-2
Pa-A	10.81	9.68	9.61	8.52	10.54	8.74	9.47	8.93	7.71	7.68
PA-M	16.16	15.69	16.80	10.80	18.45	15.69	18.13	15.75	15.42	11.22
Zi-A	-	-	-	-	-	-	-	-	-	-
Zi-M	22.22	22.04	21.97	28.68	15.72	20.74	19.84	16.38	18.03	22.76
Ma-A	-	-	9.58	6.55	-	8.54	9.13	10.93	4.01	21.77
Ma-M	-	-	-	-	-	-	-	-	-	-
EI-A	10.82	12.09	7.73	14.24	7.95	7.18	8.31	9.20	10.15	8.45
Bi-A	-	18.39	8.55	10.65	11.76	7.82	12.42	8.77	9.11	10.32
Ro-A	-	-	-	-	-	-	-	-	2.49	4.45

Average EQAP CV%	Allowable CV%	CCV%	Indian CCV%
9.2	5.0	17.0	17.3

7.68	7.71	8.52	8.74	8.93	9.47	9.61	9.68	10.54	10.81
------	------	------	------	------	------	------	------	-------	-------

CV% OF EQAP : ALP

Kit	87-1	87-2	88-1	88-2	88-3	89-1	89-2	89-3	90-1	90-2
Pa-A	15.31	11.95	13.10	12.56	11.30	10.86	11.43	10.53	10.33	11.17
PA-M	18.43	15.02	16.91	15.15	15.16	16.92	13.79	14.42	17.12	15.20
Zi-A	-	-	-	-	-	-	-	-	-	-
Zi-M	-	-	38.34	37.62	37.20	51.14	43.48	39.69	40.86	37.09
Ma-A	-	-	25.64	18.98	-	20.65	5.62	14.86	17.88	27.90
Ma-M	-	-	-	-	-	-	-	-	-	-
EI-A	-	27.67	18.16	16.56	28.11	18.51	15.96	18.15	18.66	12.45
Bi-A	-	20.64	15.95	19.31	16.47	18.82	20.23	13.39	16.09	17.39
Ro-A	-	-	-	-	-	-	-	-	38.47	35.39

Average EQAP CV%	Allowable CV%	CCV%	Indian CCV%
11.9	7.5	15.5	15.5

10.33	10.53	10.86	11.17	11.30	11.43	11.95	12.56	13.10	15.31
-------	-------	-------	-------	-------	-------	-------	-------	-------	-------

CV% OF EQAP : LD

Kit	87-1	87-2	88-1	88-2	88-3	89-1	89-2	89-3	90-1	90-2
Pa-A	14.32	10.33	11.00	11.81	11.38	11.49	12.43	11.65	10.27	10.66
PA-M	28.33	16.69	15.47	15.71	18.95	16.61	18.45	14.33	17.16	17.41
Zi-A	-									
Zi-M	-	-	-							
Ma-A	-									
Ma-M	-									
EI-A	17.3	32.64	22.40	13.82	9.02	8.49	7.86	15.97	27.69	36.22
Bi-A	-	-	23.25	8.79	-	10.35	9.88	8.86	10.50	7.01
Ro-A									3.17	4.03

Average EQAP CV%	Allowable CV%	CCV%	Indian CCV%
11.5	5.0	8.0	15.5

10.27	10.33	10.66	11.00	11.38	11.49	11.65	11.81	12.43	14.32
-------	-------	-------	-------	-------	-------	-------	-------	-------	-------

BILE PIGMENTS DETERMINATION

BILIRUBIN DETERMINATION

- **DIRECT SPECTROPHOTOMETRIC**
- *Bilirubin Is the Main (40-95%) Serum Pigment; Other Pigments Include Carotene, Xanthophyl Ester, Bilifuscin & Mesobilifuscin*
- *Absorbance at 454 nm*
- *Interferences due to Lipochromes, Hemolysis, Turbidity*
- *Only for Newborns*
- $A_{454} - A_{540}$

BILIRUBIN DETERMINATION

■ OXIDATION METHODS

■ *Gemelin*

■ *Marchal & Maher*

■ *Smidth*

■ *Fouchet*

■ *Harrison Spot Test*

BILIRUBIN DETERMINATION

■ DIAZOTIZATION METHODS

- Bilirubin + Diazotized Sulfanilic Acid \longrightarrow Azobilirubin
- In 1883, Ehrlich first describe this reaction by using urine sample
- In 1913, van der Berg Showed that Diazo reaction can be used for serum only after adding a solubilizer or accelerator
- In 1937, Malloy and Evelyn developed the first clinically useful methodology for quantitation of bilirubin in serum samples using classic diazo reaction with a 50% methanol as an accelerator
- In 1938, Jendrasic and Grof described a method using the diazo reaction with caffeine-enzoate-acetate as an accelerator
- In the early 1980s, it was recognized that total bilirubin is not equal to conjugated bilirubin plus unconjugated bilirubin

BILIRUBIN DETERMINATION

■ Malloy & Evelyn Method

- Reaction takes place at pH 1.2 in which azobilirubin has a red-purple color with a maximal absorption at 560 nm
- Uses methanol as accelerator, which can cause turbidity with proteins

BILIRUBIN DETERMINATION

■ Jendrassik & Grof Method

- By adding alkaline tartarate, absorbance spectrum of azobilirubin to a more intense blue color that is less subject to interfering substances in the sample and is measured at 600 nm
- This method is slightly more complex, but has the following advantages over the Malloy-Evelyn method :
 - Not affected by pH changes
 - Insensitive to a 50-fold variation in protein concentration
 - Maintains optical sensitivity even at low bilirubin concentrations
 - Has minimal turbidity and a relatively constant blank
 - Is not affected by hemoglobin up to 750 mg/dL

TYPES OF BILIRUBIN

- **Bond To Glucoronide (Conjugated)**
- *Monoglucoronide* → β
- *Diglucoronide* → γ
- **Bond to Albumin**
- *Noncovalently (Unconjugated)* → α
- *Covalently Bilirubin* → δ

BILIRUBIN DETERMINATION

■ SAMPLE

- Serum or plasma can be used
- Serious loss of bilirubin occurs after exposure to fluorescent and indirect and direct sunlight
- If left unprotected from light, bilirubin values may reduce by 30-50% per hour
- If serum or plasma is separated from the cells and stored in dark, it is stable for 2 days at room temperature, 1 week at 4°C, and indefinitely at -20°C

DETECTION OF BILIRUBIN IN URINE

■ Shake Test

■ Oxidation Methods

- 1) Gemelin (Nitric acid)
- 2) Marshal (Sodium nitrite in acid)
- 3) Smith (Iodide alcohol)
- 4) Fouchet (Ferric chloride in acid)
- 5) Harison pot test (Ferric chloride and Barium sulfate)

■ Diazotization Methods

- 1) Reagent strip (2,4-Dichloroanilin)
- 2) Hanter Diazotization (sodium nitrite and sulfanilic acid)
- 3) Ictotest (para-nitrobenzene diazonium)

BILIRUBIN DETECTION

■ False Positive Results

- 1) *Drugs such as Phenazothiazine, Chlorpromazine,*
- 2) *Indole and indican*

■ False Negative Results

- 1) *Exposure to light*
- 2) *Hydrolysis of bilirubin diglucoronide*
- 3) *Ascorbate*
- 4) *Nitrite*

CV% OF EQAP : Total Bilirubin

Kit	87-1	87-2	88-1	88-2	88-3	89-1	89-2	89-3	90-1	90-2
Pa-A	22.68	15.55	14.58	16.37	19.24	13.80	17.69	18.55	17.58	13.06
PA-M	29.87	14.61	22.44	19.73	18.30	22.65	25.08	17.00	32.86	18.13
Home	-	19.16	16.67	-	-	16.77	45.4	45.58	22.74	-
Zi-M	-	-	-	-	-	-	-	-	-	-
Ma-A	-	-	-	-	-	-	-	-	-	-
Ma-M	-	-	-	-	-	-	-	-	-	-
EI-A	-	-	26.24	23.13	27.37	12.55	14.57	15.29	12.50	11.17
Bi-A	-	-	17.45	18.07	27.52	10.30	18.98	12.95	13.89	11.67
Ro-A	-	-	-	-	-	-	-	-	13.13	8.45

Average EQAP CV%	Allowable CV%	CCV%	Indian CCV%
16.9	10.0	19.2	19.2

13.06	13.80	14.58	15.55	16.37	17.58	17.69	18.55	19.24	22.68
-------	-------	-------	-------	-------	-------	-------	-------	-------	-------

CV% OF EQAP : Direct Bilirubin

Kit	87-1	87-2	88-1	88-2	88-3	89-1	89-2	89-3	90-1	90-2
Pa-A	18.52	13.28	17.85	20.49	26.76	16.99	29.21	27.28	29.96	24.06
PA-M	71.32	36.46	41.54	40.24	36.89	23.92	36.15	39.68	32.34	36.72
Home	-	51.50	46.77	-	-	43.66	-	61.09	39.50	65.17
Zi-M	-	-	-	-	-	-	-	-	-	-
Ma-A	-	-	-	-	-	-	-	-	-	-
Ma-M	-	-	-	-	-	-	-	-	-	-
EI-A	-	-	40.32	37.03	-	21.79	25.43	44.71	20.18	29.69
Bi-A	-	-	-	-	-	-	-	-	-	-
Ro-A	-	-	-	-	-	-	-	-	12.34	9.87

Average EQAP CV%	Allowable CV%	CCV%	Indian CCV%
22.4	10.0	19.2	19.2

13.28	16.99	17.85	18.52	20.49	24.06	26.76	27.28	29.21	29.96
-------	-------	-------	-------	-------	-------	-------	-------	-------	-------

