ASCORBIC ACID (VITAMIN C)

For Research only **50 Tests**

INTRODUCTION :

L-Ascorbic acid, a water-soluble vitamin, is a modified hexose with strong acidic and reducing properties. In solution, ascorbate is readily oxidised by air to dehydro –L-ascorbate, especially in the presence of metallic ions such as Cu^{2+}. Both forms are biologically active and are interconvertible in the body. The rate of oxidation of ascorbate in vitro increases with increasing pH but above pH 5, dehydroascorbate also undergoes further oxidation, ring opening and loss of biological activity. The mode of action of ascorbate is unclear. It is necessary for the development of cartilage, bone and dentine. The vitamin is a cofactor for protocollagen hydroxylase which is involved in the hydroxylation of proline during connective tissue maturation. L-Ascorbate is also involved in relation to Fe(II)-enzyme systems in the metabolism of tyrosine and dihydroxyphenylalanine and in microsomal drug metabolism. The best dietary sources of the vitamin are citrus fruits, berries, tomatos, raw cabbage other green vegetables. Losses during processing and cooking can be considerable. In man, ascorbate and dehydro - ascorbate are readily absorbed from the stomach and ileum into the circulation. Ascorbate diffuses passively into many cells but platelets, adrenal and retinal cells posses active transport mechanisms.

PRINCIPLE :

Redox reaction of ascorbate with 2,6-dichlorophenol indophenol in acid solution involves reduction of this dye to a colourless leucobase while ascorbate is oxidized to dehydroascorbate. In blood and urine the vitamin is mainly present as ascorbate in fresh samples. Immediate redox reaction or preservation of the ascorbate in acid is important.

REAGENTS :

1.	Buffer	100 mM / L
2.	2,6- dichlorophenol-indophenol (DCPIP)	1. 0 mM / L

PROCEDURE :

	Blank ml	Sample ml
Sample(fresh)	-	0.1
D.Water	0.1	-
Buffer (R1)	0.5	0.5
DCPIP (R2)	1.0	1.0

Mix well. Measure the absorbance of sample (A_{Sample}) and blank (A_{blank}) against d. water at 520 nm. Linearity up to 300 mg / L. Color stable for one hour.

CALCULATION :

Ascorbate in sample (mg / L)

$$= {}^{A}_{Blank} - {}^{A}_{Sample} \times 410 \times \text{dilution factor}$$

REFERENCE RANGE

Leucocytes :

The reference range for the buffy layer is 21 to 57 µg / 10^8 leucocytes and for the leucocytes it is 11 to 21 µg / 10^8 leucocytes .

SAMPLES

Leucocytes :

Heparinized blood is mixed well, then allowed to sediment for 45 minutes at 4ºC followed by removal of the supernatant leucocyte rich plasma. Low speed centrifuge (1000 rpm) for 10 minutes sedment leucocytes . After resuspension of leucocytes in known volume of buffer (R1), ascorbate is measured at once .

Plasma :

Blood can be collected into heparin, EDTA or oxalate. The plasma is best separated and measured at once .

Urine :

The urine must be measured fresh, that is within a few minutes of being passed The daily output of ascorbate, usually about 20 to 30 mg, is about half the daily intak. The minimum intake that protects against scurvy is about 60 mg. In deficiency states, urinary ascorbate is almost absent. The 24 h excretion is not a good index of the deficiency state and a saturation test is preferable.

Plasma :

Plasma ascorbate levels are of limited value in assessing the severity of deficiency of this vitamin. On an adequate diet, plasma ascorbate levels are between 4 and 20 mg / L, and frequently 8 to 14 mg / L. Values below 2 mg /L. suggest marked deficiency. Ascorbate disappears more rapidly from the plasma than from the cells when a diet is low in vitamin C and the leucocytes appear to be the last to be depleted. The determination of leucocytes ascorbate has been used as a better index of sever deficiency .

REFERENCE:

Harris, L.J, and Ray,S.N. (1935) Lancet 1, 71, 462 .

El – Aaser, A.A. (unpublished)

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

ASCORBIC ACID (VIT. C)

Colorimetric Method

+4 to +8ºC	**50 Tests**
CAT. No.	AS 25 15

FOR RESEARCH USE ONLY

REAGENTS

R1	Buffer	**25 ml**
R2	DCPIP	**50 ml**

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