14. LITERATUR

Böhn U et al. (2003) Rationelle Diagnostik in der Orthomolekularen Medizin. Hippokrates Verlag, Stuttgart

Esteve MJ, Farre R, Frigola A, Garcia-Cantabella JM (1997) Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. J Chromatogr B Biomed Sci Apll. 24;688(2):345-9.

Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, 4th ed. Saunders: Philadephia, 2006:1107.

Verwendete Symbole:

	Temperaturbegrenzung	REF	Bestellnummer
IVD	In-Vitro-Diagnostikum	Σ	Inhalt ausreichend für <n> Prüfungen
	Hersteller		Verwendbar bis
LOT	Chargenbezeichnung		

Vitamin C

For colorimetric determination of Vitamin C in Li-heparine-plasma, serum and urine

Valid from 11.04.2012

REF K 4000 +2°C +8°C Σ 96 -20°C CTRL 1 CTRL 2 IVD CE

Immundiagnostik AG
Stubenwald-Allee 8a
64625 Bensheim, Germany
www.immundiagnostik.com

Content

1. INTENDED USE

This colorimetric microtiter plate assay is suitable for the determination of vitamin C (ascorbic acid) in Li-heparine-plasma, serum and urine. For *in vitro* diagnostic use only.

2. INTRODUCTION

Vitamin C (ascorbic acid), being a **part of the antioxidative defense system**, is found in both the cytosol and extracellular spaces. Depending on the concentration and the availability of transitional metals, it has antioxidative as well as prooxidative features. The antioxidative effect dominates, especially in extracellular space. Since it acts through formation of semi-dehydro-ascorbate and dehydro-ascorbate respectively, as an electron donor transferring hydrogen to acceptor substances by reversibility, ascorbic acid has strong reducing effects.

Vitamin C contributes to the antioxidative defense system in two different ways: it reacts with reactive oxygen species, especially peroxide radicals, and regenerates a-tocopherol (vitamin E). Vitamin C also has a pro-oxidative effect in combination with transition metals. It catalyses the reduction of Fe^{3+} to Fe^{2+}. The created bivalent iron ions react faster with H_2O_2. Therefore, the formation of $OH^{\cdot}$-radicals is supported through the Haber-Weiss-Reaction.

Due to the very small concentration of free transition metals in biological tissues, the antioxidative features are predominant. **As a result of increased oxidative stress, the level of vitamin C is reduced in various syndromes**, e.g. the level of vitamin C in blood from **HIV positive patients** is significantly lower. The content in blood plasma falls from 75.7 µmol/l to 40.7 µmol/l. **Smoking** causes a high consumption of vitamin C in the blood plasma. Protein thiols are oxidised and after the Vitamin C pool has been depleted, lipid peroxidation begins.

Indications

- Determination of vitamin C status
- Monitoring infusion therapy
- Monitoring of oral vitamin C substitution (checking the individual capacity of gastro-intestinal vitamin C resorption)

3. PRINCIPLE OF THE TEST

In serum and plasma vitamin C is found as ascorbic acid as well as its oxidized form, dehydro-ascorbate. Both forms are biologically active. In our vitamin C assay, an oxidation is induced prior to analysis so that both forms are measured. A dose response curve of the absorbance unit (optical density, OD at 492 nm) vs. concentration is generated, using the values obtained from the standard. The concentration of the patient sample is determined directly from the linear standard curve.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 4000FR	PREC	Precipitation reagent	15 ml
K 4000LSGA	SOL A	Reagent solution A	7 ml
K 4000LSGB	SOL B	Reagent solution B	1 ml
K 4000LSGC	SOL C	Reagent solution C	1 ml
K 4000AC	STOP	Sulfuric acid	20 ml
K 4000ST	STD	4 Standards (lyophilized)	each 4 x 400 µl
K 4000KO1 K 4000KO2	CTRL1 and CTRL2	Control 1 and 2 (lyophilized)	each 4 x 250 µl
K 4000MTP	PLATE	Microtiter plate (MTP)	12 x 8 wells
K 4000FOL	FOL	Microtiter plate coverfoil	2

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Precision pipettors and disposable tips to deliver 20 - 200 µl and 100 - 1000 µl
- A multi-channel dispenser or repeating dispenser
- 1.5 ml Eppendorf cups
- 15 ml Tubes (e.g. Falcon)
- Horizontal microtiter plate shaker
- Centrifuge suitable for 15 ml tubes at 10 000 x g
- Vortex-Mixer
- Incubator for 37 °C
- Microtiter plate reader at 490 - 520 nm (reference wave length 610 - 630 nm)
- A suitable place mat when working with solution A, because solution A contains dye which might be difficult to clean off plastic surfaces

6. PREPARATION AND STORAGE OF REAGENTS

- All reagents are stable at 2-8°C up to the expiry date stated on of the label; **CTRL1** and **CTRL2** (control 1 and control 2) at -20°C.
- **STD** (standards) must be reconstituted with 400 µl of bidist. water, **CTRL1** and **CTRL2** (control 1 and control 2) with 250 µl of bidist. water. Allow the vial content to dissolve for 10 minutes. Reconstituted standards and controls are not stable.

Please note: Samples should be kept cool and light-protected. Samples can be measured within 24 hours after blood withdrawal.

7. PRECAUTIONS

- For *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- STOP (stop solution) is composed of sulfuric acid, which is a strong acid. It must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- PREC (precipitating reagent) contains acid and must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on the kit label.

8. SAMPLE AND REAGENT PREPARATION

- Pipet 200 µl fresh collected Li-heparine-plasma sample, serum, **STD** (standards) or **CTRL1** and **CTRL2** (control 1 and control 2) in Eppendorf cups, respectively, and add 200 µl **PREC** (precipitation reagent)
- Urine samples must be diluted 1:4 before analysis (e. g. 250 µl urine + 750 µl aqua dist.). The dilution factor must be considered when calculating the concentration.
- Vortex well
- Centrifuge at 10000 x g for 30 min
- **Preparation of the working solution:**
  To run a complete **PLATE** (microtiter plate):

  Add 600 µl of each, **SOL B and C** (reagent solution B and C) to 6 ml of **SOL A** (reagent solution A).

To run assay more than once, prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.

Please note:
SOL A (solution A) contains dye which might be difficult to clean off plastic surfaces. It is therefore recommended to use a suitable place mat when working with **SOL A** (solution A.)

9. ASSAY PROCEDURE

Test procedure

1.	Add **2 x 100 µl** of the **supernatants** of **STD** (standard), **CTRL1** and **CTRL2** (control 1 and control 2) or **samples** into the **PLATE** (microtiter plate) wells in duplicates
2.	Add **50 µl** of the freshly prepared **working solution** in the wells
3.	Cover the **PLATE** (microtiter plate) with foil and incubate **for 3 h at 37 °C**
4.	Add **150 µl** of **STOP** (STOP-solution) in the wells
5.	Shake **PLATE** (microtiter plate) on a horizontal shaker at **room temperature for 20 min** (without any foil cover). An orange precipitate can be formed. The precipitate can be dissolved by repeatedly (2-3 times) drawing up the solution with the pipette.
6.	Determine the absorption at 492 nm or 520 nm against 620 nm as a reference

10. RESULTS

A dose response curve of the absorbance unit (optical density, OD at 492 nm) vs. concentration is generated, using the values obtained from standard. The concentration of patient samples is determined directly from the linear standard curve.

It is recommended to use a point-to-point-calculation.

Please refer to the QC data sheet for the concentration of standards (STD) and controls (CTRL).

11. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values

Normal range
4 - 15 mg / L
(Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, 4th ed. Saunders: Philadephia, 2006:1107.)

12. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay-Variation
The precision (intra-assay variation) was calculated from 14 replicate determinations on one sample.

Intra-Assay CV n= 14

Sample	Vitamin C Mean Value [mg/l]	Intra-Assay CV [%]
1	10.49	6.03

Inter-Assay-Variation

The total precision (inter-assay variation) of the vitamin C test was calculated from data obtained with four samples on three different days.

Inter-Assay CV n= 3

Sample	Vitamin C Mean Value [mg/l]	Inter-Assay CV [%]
1	13.8	5.06
2	4.5	16.74
3	9.3	11.75
4	12.0	1.80

Recovery

Two plasma samples, one with a low vitamin C content (5.1 mg/l) and a second with a high vitamin C content (16.6 mg/l), was mixed 1:1 and analyzed.

Recovery n=1

Sample	Vitamin C Expected [mg/l]	Vitamin C Measured [mg/l]	Recovery [%]
1	10.9	11.9	109.2

Sensitivity

The detection limit was defined as B_0 + 3 SD. The zero-standard was measured 9 times.

Sample	Vitamin C Mean Value [OD]	Standard variation	Detection limit [mg/l]
1	0.069	0.0021	0.7

Sample values

Heparin plasma, Serum and Urine

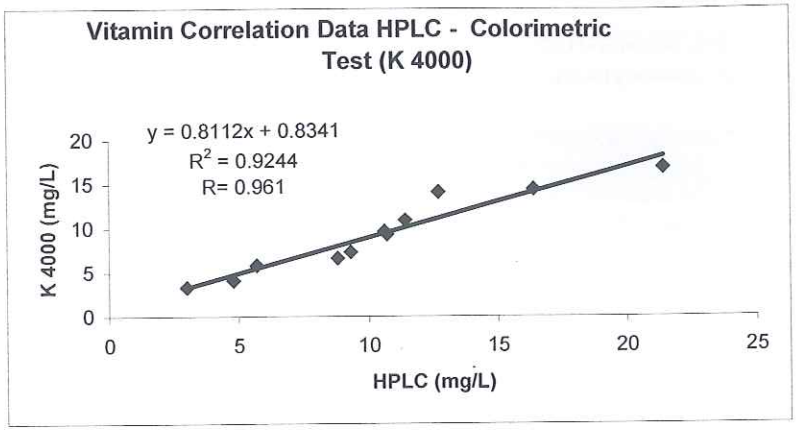
Samples from obviously healthy volunteers were evaluated for the presence of Vitamin C in this assay.

Sample	Mean (mg/L)	Range (mg/L)	Standard Deviation (mg/L)
Heparin plasma (n=6)	14.8	10.4 – 21.4	4.4
Serum (n=6)	15.5	11.7 – 19.3	3.6
Urine (n=6)	60.6	11.5 – 177.4	43.2

Correlation data between the colorimertric microtiter plate test and HPLC

Eleven samples were measured using the colorimertric microtiter plate test and HPLC. The results are shown in the table:

Sample	Vitamin C - HPLC [mg/l]	Vitamin C - Colorimetric test [mg/l]
1	11.4	10.9
2	9.3	7.3
3	5.7	5.8
4	8.8	6.6
5	10.7	9.3
6	21.4	16.9
7	4.8	4.1
8	10.6	9.6
9	16.4	14.4
10	12.7	14.1
11	3.0	3.3



13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Do not use the reagents after the date of expiry stated on the label.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.

14. REFERENCES

Böhn U et al. (2003) Rationelle Diagnostik in der Orthomolekularen Medizin. Hippokrates Verlag, Stuttgart

Esteve MJ, Farre R, Frigola A, Garcia-Cantabella JM (1997) Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. J Chromatogr B Biomed Sci Apll. 24;688(2):345-9.

Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, 4th ed. Saunders: Philadephia, 2006:1107.

Used Symbols:


Store at

Catalog Number

In Vitro Diagnostic Device

No. of tests


Manufacturer


Use by

Chargennummer