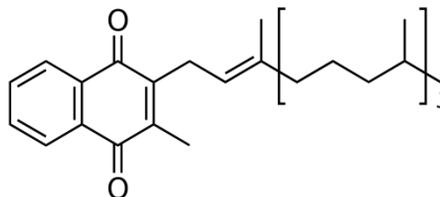


Quantitative determination of vitamin K1 in serum by LC-FLD

Vitamin K1, also known as phylloquinone, is synthesized by plants.

Serum transport of these lipophilic compounds to their target tissues takes place via lipoproteins.

The classic functions of vitamin K1 is its activity in the production of blood-clotting proteins. Animals may also convert it to vitamin K2.

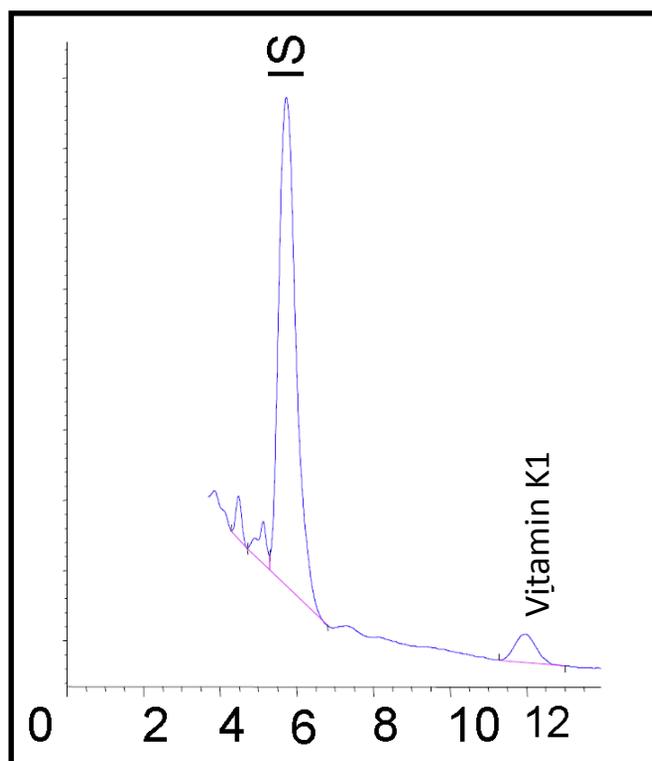


Vitamin K1 (Phylloquinone)

Serum Vitamin K1 concentration is typically 0.05-3 ng/ml. Deficiency is seen with malabsorption (Cystic fibrosis, Ulcerative colitis, and pancreatic insufficiency).

Vitas AM-093 separates Vitamin K1 from matrix components and by on-line electrochemical reduction, fluorescence is induced before detection by a fluorescence detector. The method is based on protein precipitation, on-column SPE, on-line electrochemical reduction and RP-HPLC-FLD.

Vitamin K1 (and internal standard in plasma



Method details:

- Technique: RP-HPLC-FLD
- Sample Matrix: Plasma, serum
- Species: All
- Anticoagulant: All
- Required sample volume: 150 μ L
- Shipping: Dry Ice, protect from light
- Method Range : 0.05-3.0 ng/ml
- LOD: 0.01 ng/ml
- Precision: 7.0 %
- Accuracy: KEQAS

Vitas is a Norwegian GMP certified chemical analysis contract lab, with 20 years experience in providing a high quality, custom chromatographic analytical service based on cutting-edge knowledge and technology.

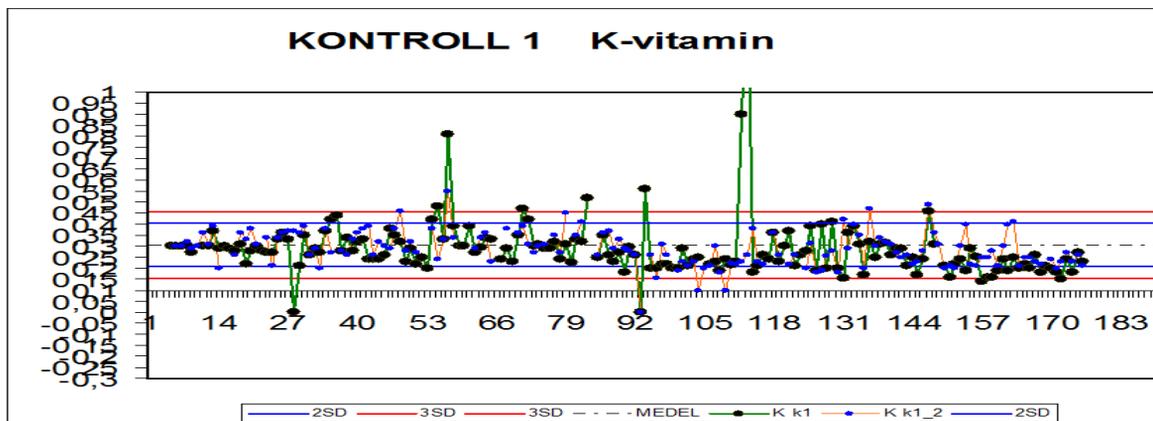
Additional information:

• Experience & Expertise

- Analysis of Vitamin K1 in human serum is regarded as very challenging due to the low normal range (0.05-3 ng/ml). Not many laboratories are able to keep a robust high quality routine method for this biomarker.
- Vitas has performed the quantitative determination of Vitamin K1 in human serum with AM-093 for close to 20 years.
- Currently the number of samples analysed are 1300 per month.
- Vitas receive samples for Vitamin K1 analysis from several customers, the largest one being “Først Medisinske laboratorium” in Oslo. Først mainly receives samples from the Norwegian primary health care.
- Vitas has over many years optimised the method AM-093 so that is today delivers reproducible results from as little as 150 µL and has a LOQ of 0.05 ng/ml. The analytical setup is unusually complex for a routine method, but this complexity is necessary to achieve the low LOQ from low sample volumes.

• Quality assurance

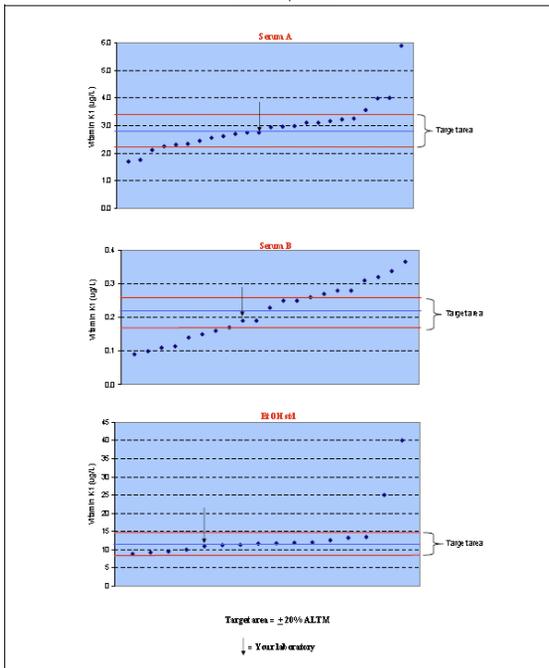
- Vitas has a comprehensive and stringent regime for quality assurance.
- In each series of sample there are serum based controls at two levels early and late in the sequence. The obtained values are then plotted in a control plot and trended and used to release the samples analysed in the same series.
- An example of a control plot for Vitamin K1 is shown below.



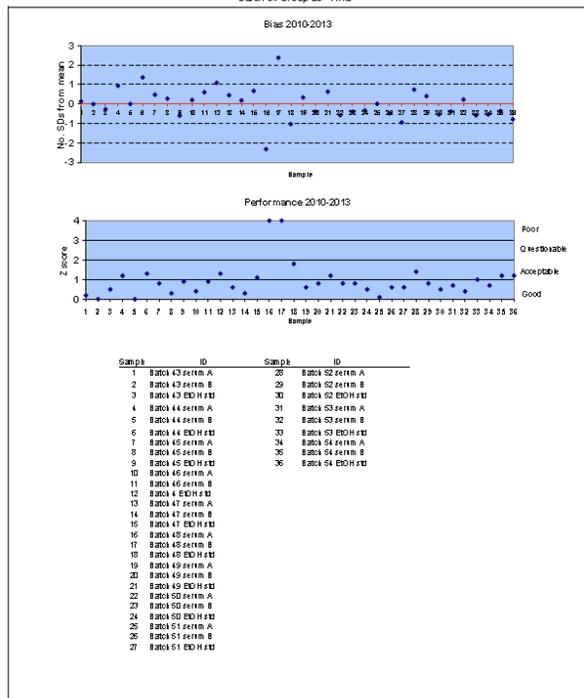
Additional information page 2:

- External quality assurance Scheme (EQAS)**
 - Vitas has for many years participated in a EQA for Vitamin K in human serum and plasma called KEQAS.
 - KEQAS is an international scheme that monitors and reports on the accuracy of vitamin K1 (phylloquinone) analysis. KEQS is a member of UKNEQAS and is CPA (EQA) accredited.
 - Members receive 3 batches of 3 blind samples each year and are required to analyse them during specific periods over the course of the year. When results are returned they are subjected to statistical analysis including an outliers test and a Z scoring system. The target for accuracy of analysis is currently set at 20 % from the All Laboratories Trimmed Mean (ALTM) and this is the standard by which results are judged good, acceptable, questionable or unacceptable. Members then receive comprehensive reports detailing their performance. The result from the latest round is shown below (left) together with the historical performance of Vitas (Lab28) from 2010-2013. The full reports are attached. Vitas has performed very well.

KEQAS
Batch 56 Group 28 - Final



KEQAS
Batch 54 Group 28 - Final



Publications - VitK

1. [Vitamin K2 in different bovine muscles and breeds](#) Rune Rødbotten, Thomas Gundersen, Cees Vermeer and Bente Kirkhus
Meat Science, 22. January 2014, <http://dx.doi.org/10.1016/j.meatsci.2014.01.005>
2. [Drevon CA, Henriksen HB, Sanderud M, Gundersen TE, Blomhoff R.](#)
Biological effects of vitamin K and concentration of vitamin K in Norwegian food. Tidsskr Nor Lægeforen. 2004 Jun 17;124(12):1650-4. Review.
3. [Vitamin K1 and 25\(OH\)D are independently and synergistically associated with a risk for hip fracture in an elderly population: A case control study](#) ☆ Anne C. Torbergsen, Leiv O. Watne, Torgeir B. Wyller, Frede Frihagen, Knut Strømsøe, Thomas Bøhmer, Morten Mowe, doi:10.1016/j.clnu.2014.01.016

References – Page 1

In addition to performing this analysis every day for 20 years Vitas has been the preferred subcontractor in numerous projects. Some of these are referred below.

Fürst Medisinsk Laboratorium, Oslo

Reference:

Marie Buchmann

Medical Director

Phone 22 90 95 53

E-mail: msbuchmann@furst.no

Vitas has performed Vitamin ADEK1 for close to 20 years. In total several hundred thousand samples.

NB! Please note that Fürst is also likely to reply to this tender

ULSAM

LSAM is a unique, ongoing, longitudinal, epidemiologic study based on all available men, born between 1920 and 1924, in Uppsala County, Sweden. The men were investigated at the ages of 50, 60, 70, 77, 82 and 88 years. Full screening and official registry data are available in our databases and more data is continuously added.

<http://www2.pubcare.uu.se/ULSAM/>

Vitas performed multiple biomarkers in several thousand samples including Vitamin K1, retinol, 25OH-Vitamin D, and RBP.

Reference:

Professor Håkan Melhus,

Uppsala Universitet

Phone: +46- 018-611 4950

E-mail: Hakan.Melhus@medsci.uu.se

NOREPOS

NOREPOS (Norwegian Epidemiologic Osteoporosis Studies) is a national research collaboration network of researchers from five different scientific institutions across Norway. We perform epidemiologic research in the field of osteoporosis. <http://norepos.b.uib.no/>

Vitas performed the analysis of of Vitamin ADEK1 in 2300 samples.

Reference:

Professor Grethe Tell

Universitetet i Bergen

Phone: 55588522

E-mail: Hakan.Melhus@medsci.uu.se

Reference:

Kristin Holvik

Researcher at Norwegian Institute of Public Health

Phone: 21 07 83 97

E-mail: Kristin.Holvik@fhi.no

References - page 2

UIO Framework Agreement

Vitas have a frame agreement for services related to multiple biomarker analysis including Vitamins ADEK. The agreement was entered into in 2010 and was renewed in 2014 for 4 additional years.

<https://www.doffin.no/Notice/Details/2013-204137>

Reference:

Hans Tore Brekke

Seniorrådgiver - Seksjon for innkjøp - Universitete ti Oslo

Tel. + 47 22 85 81 16

Mob. +47 481 48 282

EU Projects

From 2011 to 2015, Vitas have been partner in four EU projects working alongside scientists and researchers from prestigious academic institutions, other SMEs and larger industrial partners. Vitas role in these projects is method development, validation and analysis if biomarkers from biological samples.

We mention two here:

1. EPIC-CVD – University of Cambridge - <http://www.epiccvd.eu/>

This project was chosen as it demonstrates Vitas ability to do the analysis from minute sample volumes and in a high throughput manner. This is the largest study ever performed om Vitamin C and carotenoids. We are proud of the fact that Vitas was chosen as partner by this very prestigious academic institution.

- EPIC-CVD is coordinated by Prof. John Danes, Head of the 330-person Department of Public Health and Primary Care at the University of Cambridge, UK.
- The European Prospective Investigation into Cancer and Nutrition (EPIC) study is a Europe-wide prospective cohort study of the relationships between diet and cancer, as well as other chronic diseases, such as cardiovascular disease. With over half a million participants, it is the largest study of diet and disease to be undertaken.
- In this project Vitas has been selected to perform the major task in the project. Measuring Vitamin D, carotenoids and Vitamin C in 55 000 samples. The available sample volumes are minute. In this case 50, 30 and 25 µL. The throughput is about 4000 sample /month.

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Institute of Public Health
University of Cambridge

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Reference:

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References - page 3

2. Nutritech – TNO – Holland -

This project was chosen as it demonstrates Vitas ability to do deal with very complex projects. Also in this project sample volumes are minute and in a high throughput manner. In addition there are twelve nutritional biomarkers to be analysed from plasma/serum. Vitas also perform comprehensive lipidomics and targeted proteomics. The number of samples range from some hundred to several thousand depending on marker.

2. Application of new technologies and methods in nutrition research the example of phenotypic flexibility (NUTRITECH)

- Nutritech is organised by TNO, Holland and have 22 partners in total.
- NutriTech will build on the foundations of traditional human nutrition research using cutting-edge analytical technologies and methods to comprehensively evaluate the diet-health relationship and critically assess their usefulness for the future of nutrition research and human well-being. Technologies include genomics, transcriptomics, proteomics, metabolomics, laser scanning cytometry, NMR based lipoprotein profiling and advanced imaging by MRI/MRS.
- Vitas Contribute with biomarker analysis from blood, muscle and adipose tissue biopsies.
- **Biomarkers:**

- Vitamin C
- Homocysteine
- Pyridoxal-5'-phosphate (VitB6)
- 4-pyridoxic acid (VitB6)
- Folic acid (VitB9)
- Methyl malonic acid (MMA)
- Retinol
- 25hydroxy vitamin D
- Vitamin E – alfa-tokoferol
- Vitamin K1 and K2 (MK4)
- Fatty acid pattern
- Carotenoids
- Lipidomics
- Interleukines
- CRP
- C-peptide
- ICAM-1
- VCAM
- MCP-1
- Adiponectine
- TNFa
- E-Selectine

References

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