



UKNEQAS for Vitamin K



Participants' Manual

Nutristasis Unit
Viapath
St. Thomas' Hospital
London SE1 7EH
Email: david.card@viapath.co.uk
Telephone: +44 (0) 20 7188 6815
www.keqas.com

Version 14.0
2017



7586

The KEQAS Participants' Manual is valid for one year. KEQAS management perform regular reviews and if major updates or changes in procedure are required, a fully revised version will be completed. Participants must use a current, authorised version of this document from www.keqas.com

1 Table of Contents

2	Introduction	3
3	Aims and Objectives	4
4	Scope, eligibility and support	5
5	Terms, conditions and expectations of participation	6
6	Scheme organisation	7
6.1	Staff	7
6.2	Steering Committee	7
7	Samples	9
7.1	Source of samples	9
7.2	Preparation of samples	9
7.3	Homogeneity testing	9
7.4	Types of samples	9
7.5	Storage	10
7.6	Distribution and packaging	10
7.7	Infection	10
8	Reporting results	11
8.1	Transmission of results	11
8.2	Performance criteria	11
8.3	Unacceptable performance	12
8.4	Report format	12
9	Communication with participants	13
9.1	Channels of communication	13
9.2	How to apply for participation	13
9.3	Appeals against performance evaluation	13
9.4	Complaints procedure	13
9.5	Confidentiality	14
10	Fees	15
11	Useful publications	16
	Appendix 1	17 - 18

2 Introduction

Vitamin K is a fat soluble vitamin required for the function of various proteins, most notably the coagulation factors II, VII, IX and X, proteins C and S, protein Z, the bone protein osteocalcin and matrix gla protein which is important in maintenance of healthy vasculature. Vitamin K₁ is the main dietary form and largest contributor to vitamin K status. The menaquinones (vitamin K₂, MK-n) are derived from bacterial colonies in the intestines or fermented foods with the exception of menaquinone-4 (MK-4), which is also found in extra-hepatic tissues. Vitamin K₃ is available as a pharmaceutical but is also formed in vivo as an intermediate in the conversion of vitamin K₁ to MK-4.

Measurement of vitamin K can be useful in a range of clinical scenarios. Low serum or plasma vitamin K₁ concentrations can indicate insufficiency or deficiency and routine measurement in higher risk populations such as those with lipid malabsorption e.g. cholestatic disease, pancreatic disease, cystic fibrosis etc. can be beneficial. Conversely measurement of vitamin K₁ 2,3-epoxide is useful for the investigation of suspected vitamin K antagonist intoxication. In research, measurement of vitamin K has applications in furthering the understanding of vitamin K function, metabolism and optimal status. Harmonisation of vitamin K measurements therefore underpins the validity of research and accuracy of medical diagnosis.

At the 10th meeting of the European Fat-soluble Vitamins Group in 1996, it was agreed that a quality assurance scheme for the determination of vitamin K should be initiated since self 'in-house' assessment of laboratory performance makes it difficult to identify systematic errors. It was decided that participation in an external quality assurance scheme would greatly assist the development and harmonisation of methods for vitamin K analyses and their application to nutritional and clinical studies. During the Federation of American Societies for Experimental Biology summer Vitamin K meeting in 1997 KEQAS welcomed its first non-European participants. Thirty groups now support KEQAS. For some, vitamin K analysis is 'occasional' or dependent on current research priorities and flexible *ad hoc* KEQAS membership is encouraged to accommodate the continued participation of these members.

At the 11th meeting of the European Fat-soluble Vitamins Group in 2000 preliminary data from KEQAS was presented entitled Vitamin K Determination: Are we talking the same language? From 1996-2000 the coefficient of variation for serum sample analysis of vitamin K₁ began to improve. Assay selectivity however was of particular concern, this being illustrated by a number of false positives following the blind analysis of vitamin K depleted serum. KEQAS reported no bias as a result of choice of methodology e.g. fluorescence or electrochemical detection and concluded that sources of variation are likely to include both systematic and random errors with likely systematic errors including the preparation and calibration of vitamin K₁ and internal standard solutions. A more detailed explanation of this investigation was published in December 2009 (*Biomedical Chromatography*, 2009; 23(12):1276-82).

In June 2007 KEQAS became officially affiliated with UKNEQAS and in November 2008 gained full accreditation as an EQA provider from Clinical Pathology Accreditation UK Ltd. In 2009 aliquots from a serum pool will be distributed for multiple replicate analyses by participants with the aim of generating a standard reference material (SRM). KEQAS SRM-001 was characterised by multiple intra-laboratory analyses and was made available in January 2010. In 2009 pilot studies began for intra-laboratory comparison of menaquinone-4, menaquinone-7 and vitamin K₁ 2,3-epoxide.

3 Aims and Objectives

- (i) Assist in the development and harmonisation of methods for vitamin K analyses and their application to nutritional and clinical studies.
- (ii) Alert laboratories if they report results that stray from the consensus of KEQAS members and provide support in rectifying analytical problems.
- (iii) Provide a forum for communication between laboratories where ideas and information can be exchanged freely.
- (iv) To improve the quality of vitamin K analysis.

4 Scope, eligibility and support

- (i) **Accreditation:** Viapath Analytics LLP, operating KEQAS, is UKAS accredited proficiency testing provider No. 7586 for proficiency testing of vitamin K₁ analysis.
- (ii) **Pilot schemes:** EQA for vitamin K₁ 2,3-epoxide is currently in the pilot phase. In 2009 a pilot scheme for vitamin K₂ (MK-4 and MK-7) was initiated.
- (ii) **Eligibility for participation:** KEQAS is open to any group that measures any of the K vitamers supported by this scheme.
- (iii) **Support:** KEQAS is available to participants for the provision of technical advice. Contact details can be found on page 1 of this document.

5 Terms, conditions and expectations of participation

5.1 General terms of participation

- (i) Group heads must officially confirm their participation in writing and agree to pay the annual fee.
- (ii) Participants outside the UK are under no obligations except in relation to the annual fee for scheme participation.
- (iii) UK clinical service laboratories must agree in writing to participate according to the conditions of the Joint Working Group (JWG) (see Appendix 1)
- (iv) For participants providing a clinical service in the UK, conditions of confidentiality are determined by the JWG.
- (v) Participants are expected to treat KEQAS samples as they would any other material for routine vitamin K determination.
- (vi) Where possible participants are expected to analyse KEQAS samples according to the scheme schedule.

5.2 Misrepresentation of KEQAS data and brand

Registered KEQAS participants are permitted to refer to participation in KEQAS in their documentation and online on condition that their participation and performance is not misrepresented. The KEQAS brand and logo should only be used on official KEQAS documentation and may not be used by any group or individual on non-KEQAS documentation or website.

Groups or individuals are not permitted to:

- Use the KEQAS logo on their documents or website.
- Indicate that their laboratory performance is satisfactory according to KEQAS when this is not the case.
- Indicate that they are participating in KEQAS when this is not the case.
- Alter or manipulate KEQAS data in a way that alters its interpretation.

Groups or individuals are permitted to:

- Use data from KEQAS participants' results reports in their documents and publications.
- Indicate that their group participates in KEQAS if this is the case.
- Use information from KEQAS publications e.g. research articles, participant communications etc.

6 Scheme organisation

6.1 Staff

Name	Position
Mr D Card	Scheme Manager
Ms K Chowdhary	Scheme Quality Manager
Dr D Harrington	Scheme Director
Dr M Shearer	Scientific Advisor
Ms P Dhamrait	Scheme Deputy Manager

6.2 Steering Committee

Name	Position
Mr D Card	KEQAS Manager Nutristasis Unit Haemostasis and Thrombosis Viapath St Thomas' Hospital London david.card@viapath.co.uk
Ms K Chowdhary	Quality Manager Haemostasis and Thrombosis Viapath St Thomas' Hospital London kaiya.chowdhary@viapath.co.uk
Dr D Harrington	Steering Committee Chairman, KEQAS Director and Consultant Clinical Scientist Nutristasis Unit Haemostasis and Thrombosis Viapath St Thomas' Hospital London dominic.harrington@viapath.co.uk
Dr L Schurgers	Scientific Advisor Department of Biochemistry Cardiovascular Research Institute (CARIM) University of Maastricht PO Box 616 6200 MD, Maastricht The Netherlands L.Schurgers@BIOCH.unimaas.nl

Dr M Shearer

Scientific advisor
Centre for Haemostasis and Thrombosis
St Thomas' Hospital
London
martin.shearer@gstt.nhs.uk

National Quality Assurance Advisory Panel
representative

Invited to attend steering committee meetings as
an observer.

7 Samples

7.1 Source of samples

Frozen, single donor, human serum is purchased from The Dutch Blood Service and is currently screened for: HIV: Anti-HIV-1/2 and HIV-NAT (RNA), Hepatitis B: HBsAg and HBV-NAT (DNA), Hepatitis C: Anti-HCV and HCV-NAT (RNA), HTLV: Anti-HTLV-I/II and Syphilis: Anti-TP.

7.2 Preparation of samples

Samples are prepared in a way that ensures homogeneity and are indexed according to the batch and serum numbers, all preparation is carried out to ISO17043 specifications that ensure uniformity and condition of samples.

7.3 Homogeneity testing

Six aliquots of each sample in their final manufactured state are analysed prior to despatch. Homogeneity is assessed by one way ANOVA analysis according to ISO17043. Homogeneity testing is subcontracted to the Nutristasis Unit, Viapath, St. Thomas' Hospital, London.

7.4 Types of samples

Below are examples of samples currently distributed by KEQAS.

Matrices for measurement of vitamin K₁:

Sample name	Preparation	Matrix
Serum (as supplied)	Protected from light	Serum
Vitamin K depleted serum	Exposed to UV light	Serum
Spiked serum	Vitamin K ₁ spiked	Serum
Ethanol Standard	Vitamin K ₁ ethanolic standard solution	Ethanol
Standard reference material	Protected from light	Citrated plasma

Pilot scheme matrices

The pilot schemes for vitamin K₂ and vitamin K₁ 2,3-epoxide use serum and ethanol spiked with ethanolic vitamin K₁ 2,3-epoxide, menaquinone-4 and menaquinone-7 to concentrations < 10 µg/L.

Standard reference material

KEQAS SRM-001 was manufactured from pooled citrated plasma with the reference value determined by multiple inter-laboratory analyses. It has a target value of 0.25 µg/L, which was designed to be analytically challenging and clinically relevant.

Ethanol standard samples

Ethanol standard samples are not used in assessment of performance. Their analysis is not mandatory and they represent a 'tool' designed for use for in assay troubleshooting, particularly the investigation of assay interference. They may be analysed in a number of ways which differ in the proportion of the extraction procedure they are taken through i.e. injected directly to the system through to undergoing the entire extraction procedure.

7.5 Storage

KEQAS samples should be stored (long term) at -70°C and protected from light and alkaline conditions at all times.

7.6 Distribution and packaging

Samples are distributed annually in December by courier, four batches are sent out each year and each batch contains two serum samples and one ethanol standard. For further details see 'KEQAS Schedule' (available at www.keqas.com).

Additional samples for K vitamers may be added as part of the pilot scheme consists of one serum sample and one ethanol standard per batch.

Packaging conforms to The United Nations guidelines specifically relating to the transport of pathological samples (UN class 6.2 and AITA 650 guidelines).

Vitamin K₁ is known to be stable at room temperature and so is not couriered on ice. Sample stability validation has been carried out and results are available at www.keqas.com. The other K vitamers are thought to have similar stability.

7.7 Infection

All human blood and serum products and samples should be regarded as possibly infectious material and handled according to local procedures.

8 Reporting results

8.1 Transmission of results

KEQAS accepts only electronic submission of results emailed to the KEQAS manager. Results must be reported using unambiguous units, which will be converted to a common unit on receipt. The standard unit used in the scheme is µg/L. Other standard mass/volume units such as ng/mL or ng/L will be excepted although µg/L is preferred. Molar units are not accepted and participants will be asked to resubmit their results.

Late results will only be accepted if the batch reports have not been completed at the time the result is received. Amended results will be accepted if they are transmitted prior to the deadline and if they are clearly marked 'amended results'.

Results should be emailed to the KEQAS manager (currently David Card) at:
david.card@viapath.co.uk.

8.2 Performance criteria

Performance is assessed only using data from serum samples, ethanolic samples are not used for these purposes.

For inclusion in the calculation of the all laboratory trimmed mean (ALTM) data must:

- (i) Be received prior to the specified batch deadline (see yearly schedule)
- (ii) Satisfy the Grubb's test for outliers (alpha= 0.05).

The ALTM represents the target concentration, Grubb's test identifies outliers that could distort the mean value for that sample. The target for results is the current standard deviation of proficiency testing (SDPT). The SDPT is calculated from the previous three years data and is responsive to changing trends in performance due to changes in e.g. methodology.

A guide to performance is shown using a Z scoring system where:

$$Z = \frac{X_i - X}{SDPT}$$

Z: Z Score

X_i: Result

X: Mean result from all labs.

Z scores are divided into the following categories and using a traffic light style classification system:

< 2 – Satisfactory – Green

2 < Z < 3 - Questionable – Amber

Z > 3 – Unsatisfactory - Red

8.3 Unacceptable performance

Underperformance is defined by Grubb's test and by the Z scoring system. If a result is defined as an outlier by Grubb's test or if a Z score is greater than three then the performance is defined as poor (single under-performance (SUP)).

Persistent under-performance (PUP) is defined as either:

Being classified as an outlier by Grubb's test or attaining a Z score greater than three for two consecutive batches.

OR

Attaining a Z score between two and three for three consecutive batches.

If a participating laboratory is classed as a persistent under-performer then they will be contacted and offered assistance by the KEQAS scheme organiser.

All SUPs and PUPs are reported confidentially to the National Quality Assurance Advisory Panel (NQAAP) through quarterly reports. All UK groups identified as PUPs are reported to the NQAAP with the identities of the poorly performing group made available to the panel if required.

8.4 Report format

Page	Contents
1	• Title Page
2	• Individual laboratories' results • Z scores • Outliers' test
3	• Results plots
4	• (Annual Report only) • Standard deviation plot • Z scores plot
5	• Information for participants

8.5 Calibrator specific mean values

Due to bias originating from commercial calibrators it has been necessary to include calibrator specific mean values for sample round analysis in KEQAS reports. Although performance is not assessed using these values, they have been included in the report in order for participants to compare their performance to other groups using the same calibrator and assess the extent to which their performance has been influenced by groups using different calibrants. Currently, based on analysis of KEQAS data, the calibrators fall in to two groups. These are i) calibrants prepared in-house from vitamin K stock solutions with concentrations determined by UV spectrophotometry and Beer's law (primary calibration technique) or by weighing; and ii) commercially available calibrants available to purchase, method of preparation unknown.

8.6 Uncertainty of the target value

Standard uncertainty is calculated and included on reports with an explanation of how uncertainty has been calculated. The target value (ALTM) for each sample and performance criteria are validated by comparison of the uncertainty of the target value to the performance target (SDPT). The uncertainty (U) of the assigned value should be calculated thus:

$$U_{\text{assigned value}} = \frac{1.25 \times \text{SD assigned value}}{\text{No participants}}$$

If the $U_{\text{assigned value}}$ is $< 0.3 \times \text{SDPT}$ then the assigned value (ALTM) and the uncertainty associated with it are within statistically reliable limits relative to the SDPT and methods for detecting poor performance based on these is robust.

9 Communication with participants

9.1 Channels of communication

E-mail is the preferred method of communication.

Electronic reminders to analyse KEQAS batches are sent at the beginning of each analysis period.

Further information is available to participants online at www.keqas.com. To get to the 'Participants Area' first click on the 'KEQAS Participants' link.

Communications are logged using Qpulse and are discussed at monthly management meetings.

9.2 How to apply for participation

Please contact the [KEQAS Manager](#), to arrange participation.

9.3 Appeals against performance evaluation

If you are unsatisfied with the performance evaluation please follow this guidance:

Contact the [KEQAS Manager](#) and request an investigation in to your performance evaluation. Please provide details of the batch number and sample that you wish to be investigated and why you think the performance evaluation you were given was not appropriate. You will be given a participant communication reference number and the issues you raise will be discussed with the scheme Director and Quality Manager. If your appeal is successful then you will be issued with an amended report. If your appeal is rejected and you are not satisfied with the explanation you were given you may ask for the issue to be raised at the next steering meeting (held twice a year) where a final decision will be made.

9.4 Complaints procedure

KEQAS has a policy in place to deal with complaints from participants. Complaints will only be treated as such if the participant states clearly that they are making an official complaint. If this is not stated then it will be treated as a correspondence, the distinction is important as a complaint triggers the official complaints procedure.

On receipt complaints are logged on Qpulse generating a reference number and, where possible action is taken locally to address the complaint immediately. All complaints are brought up at the next monthly management meeting and any actions are discussed. If the KEQAS management cannot satisfy a complaint then it is passed on to the steering committee for a final decision.

The complaints procedure is different for UK participants according to the terms of participation set out by the Joint Working Group for Quality Assurance (see Appendix 1).

9.5 Confidentiality

All information relating to KEQAS participants is treated as strictly confidential e.g. staff, address, methods etc. Participation is anonymous with participants only known by their unique identifying number, which they are assigned on joining the scheme. Data is made available to all, but the identities of the groups are only available to KEQAS management and the steering committee.

Confidentiality is maintained at all times unless it is waived by the participant, for example when details are required for technical support.

Details of performance (i.e. PUPs and SUPs) are provided to the National Quality Assurance Advisory panel (NQAAP) but the identities of participants remains confidential. If the NQAAP or any other external regulatory authority requests participant details then the participant shall be informed in writing.

10 Fees

To cover costs of materials, couriers and time an inclusive fee of £250+VAT is payable on receipt of each annual batch of KEQAS material. Invoices will be despatched within 28 days of sample dispatch. KEQAS is non-profit making and any excess funds generated are reinvested back in to the running of the scheme.

KEQAS standard reference material is available to purchase at £250+VAT for 10 x 1 mL aliquots.

11 Useful publications

In 2009 a review of KEQAS data from 2000- 2006 was published:

Card DJ, Shearer MJ, Schurgers LJ and Harrington DJ. The external quality assurance of phylloquinone (vitamin K₁) analysis in human serum. *Biomedical Chromatography*. 2009; 23: 1276-1282.

In 2013 an abstract was accepted for ISTH entitled 'Investigation of methodological sources of bias in the measurement of vitamin K₁ (phylloquinone) in human serum at endogenous concentrations'. The abstract was presented as an Eposter and circulated to all participants. It highlighted issues of positive bias in vitamin K₁ measurement caused by the use of a commercially available calibrant.

In 2015 the KEQAS steering committee published a letter in the British Journal of Haematology on the appropriate uses of pharmacological forms of vitamin K:

Card DJ, Shearer MJ, Schurgers LJ, Gomez K and Harrington DJ. What's in a name? The pharmacy of vitamin K. *Br J Haematol*. 2015: 10.1111/bjh.13828.

Appendix 1

Joint Working Group for Quality Assurance : Conditions of EQA Scheme Participation

The Joint Working Group for Quality Assurance (JWG) is a multidisciplinary group accountable to the Royal College of Pathologists for the oversight of performance in external quality assurance schemes (EQA) in the UK. Membership consists of the Chairmen of the National Quality Assurance Advisory Panels (NQAAPs), and representatives from the Institute of Biomedical Sciences, the Independent Healthcare Sector, the Department of Health and CPA (UK) Ltd.

1. The Head of a laboratory is responsible for registering the laboratory with an appropriate accredited EQA scheme.
2. The laboratory should be registered with available EQA schemes to cover all the tests that the laboratory performs as a clinical service.
3. EQA samples must be treated in exactly the same way as clinical samples. If this is not possible because of the use of non-routine material for the EQA (such as photographs) they should still be given as near to routine treatment as possible.
4. Changes in the test methodology of the laboratory should be notified in writing to the appropriate scheme organiser and should be reflected in the EQA schemes with which the laboratory is registered.
5. Samples, reports and routine correspondence may be addressed to a named deputy, but correspondence from Organisers and NQAAPs concerning persistent poor performance (red – see below) will be sent directly to the Head of the laboratory or, in the case of the independent healthcare sector, the Hospital Executive Director.
6. The EQA code number and name of the laboratory and the assessment of individual laboratory performance are confidential to the participant and will not be released by Scheme Organisers without the written permission of the Head of the laboratory to any third party other than the Chairman and members of the appropriate NQAAP and the Chairman and members of the JWG. The identity of a participant (name of laboratory and Head of Department) and the tests and EQA schemes for which that laboratory is registered (but not details of performance) may also be released by the Scheme Organiser on request to the Health Authority, Hospital Trust/Private Company in which the laboratory is situated after a written request has been received.
7. A NQAAP may, with the written permission of the Head of a laboratory, correspond with the Authority responsible for the laboratory, about deficiencies in staff or equipment which, in the opinion of the NQAAP members, prevent the laboratory from maintaining a satisfactory standard.
8. Laboratories' EQA performance will be graded using a traffic light system; green will indicate no concerns, amber poor performance, red persistent poor performance, with black being reserved for the tiny number of cases that cannot be managed by the Organiser or NQAAP and that have to be referred to the JWG. The criteria for poor performance (amber) and persistent poor performance (red) are proposed by the EQA scheme Steering Committee in consultation with the EQA Provider/Scheme Organiser and approved by the relevant NQAAP.

9. When a laboratory shows poor (amber) performance the Organiser will generally make contact with the participant in accordance with the Scheme Standard Operating Procedure for poor performance. Within 2 weeks of a laboratory being identified as a persistent poor performer (red), the Organiser will notify the Chairman of the appropriate NQAAP together with a resume of remedial action taken or proposed. The identity of a persistently poor performing laboratory (red) will be made available to members of the NQAAP and JWG. The NQAAP Chairman should agree in writing any remedial action to be taken and the timescale and responsibility for carrying this out; if appropriate, this letter will be copied to accreditation/regulatory bodies such as CPA (UK) Ltd, UKAS and HFEA who may arrange an urgent visit to the laboratory. Advice is offered to the Head of the Laboratory in writing or, if appropriate, a visit to the Laboratory from a NQAAP member or appropriate agreed expert may be arranged.

10. If persistent poor performance remains unresolved (black), the NQAAP Chairman will submit a report to the Chairman of the JWG giving details of the problem, its causes and the reasons for failure to achieve improvement. The Chairman of the JWG will consider the report and, if appropriate, seek specialist advice from a panel of experts from the appropriate professional bodies to advise him/her on this matter. The Chairman of the JWG will be empowered to arrange a site meeting of this panel of experts with the Head of the Department concerned. If such supportive action fails to resolve the problems and, with the agreement of the panel of experts, the Chairman of the JWG will inform the Chief Executive Officer, or nearest equivalent within the organisation of the Trust or Institution, of the problem, the steps which have been taken to rectify it and, if it has been identified, the cause of the problem. The Chairman of the JWG also has direct access and responsibility to the Professional Standards Unit of the Royal College of Pathologists. Should these measures fail to resolve the issues, the laboratory will be referred to the Care Quality Commission for further action.

11. Problems relating to EQA Schemes, including complaints from participating laboratories, which cannot be resolved by the appropriate Organiser, Steering Committee or NQAAP, will be referred to the Chairman of the JWG.

Joint Working Group for Quality Assurance in Pathology, August 2010.