UKNEQAS for Vitamin K

Participants' Manual

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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

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 K_1 is the main dietary form and predominant circulatory form. The menaquinones (vitamin K_2, 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 K_3 is available as a pharmaceutical but is also formed in vivo as an intermediate in the conversion of vitamin K_1 to MK-4.

Measurement of vitamin K can be useful in a range of clinical scenarios. Low serum or plasma vitamin K_1 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 K_1 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. Forty groups now support KEQAS.

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 K_1 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 K_1 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. 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 K_1 2,3-epoxide. In 2019 a new pilot scheme for the measurement of undercarboxylated prothrombin (PIVKA-II) was initiated. PIVKA-II can be used as a marker of vitamin K status or hepatocellular carcinoma.

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, enrolment and support

(i) **Accreditation:** Viapath Analytics LLP, operating KEQAS, is an ISO 17043 accredited proficiency testing provider No. 8595 for vitamin K_1 analysis.

(ii) **Pilot schemes**: EQA for vitamin K_1 2,3-epoxide is currently in the pilot phase. In 2009 a pilot scheme for vitamin K_2 (MK-4 and MK-7) was initiated.

(ii) **Eligibility for participation**: KEQAS is open to any group that measures any of the analytes supported by this scheme.

(iii) Enrolment forms are distributed annually. If contact details change this must be communicated to KEQAS management and updated on the EQALite online reporting system.

(iv) **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 and conditions 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 https://www.rcpath.org/uploads/assets/9dc66c91-95c3-4d49-b8fe0222f605d70b/Joint-Working-Group-on-Quality-Assurance-Conditions-of-EQA-Scheme-Participation.pdf).

(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 analysis.

(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 E Freke	Scheme Quality Manager
Dr D Harrington	Scheme Director
Dr M Shearer	Scientific Advisor
Prof. L Schurgers	Scientific Advisor

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 E Freke	KEQAS Quality Manager Haemostasis and Thrombosis Viapath St Thomas' Hospital London ella.freke@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 I Jennings	Statistical Advisor UK NEQAS For Blood Coagulation 3rd Floor Pegasus House 463A Glossop Road Sheffield S10 2QD i.jennings@coageqa.org.uk

Prof. 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 (Sanquin) 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 ISO 17043 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 of vitamin K_1 is assessed by one way ANOVA analysis according to ISO17043. Analysis for 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 K_1:

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
Ethanolic Standard	Vitamin K_1 ethanolic standard solution	Ethanol

Pilot scheme matrices

The pilot schemes for vitamin K_2 and vitamin K_1 2,3-epoxide use serum and ethanol spiked with ethanolic vitamin K_1 2,3-epoxide, menaquinone-4 and menaquinone-7 to concentrations < 10 µg/L. For PIVKA-II samples are made by spiking with synthetic PIVKA-II antigen or using diluted serum containing high concentrations of endogenous PIVKA-II.

Ethanolic standard samples

Ethanolic 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 -70ºC 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 ethanolic 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 ethanolic 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 K_1 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 Participant's guide on how to use the EQA*lite* service to submit results and access reports

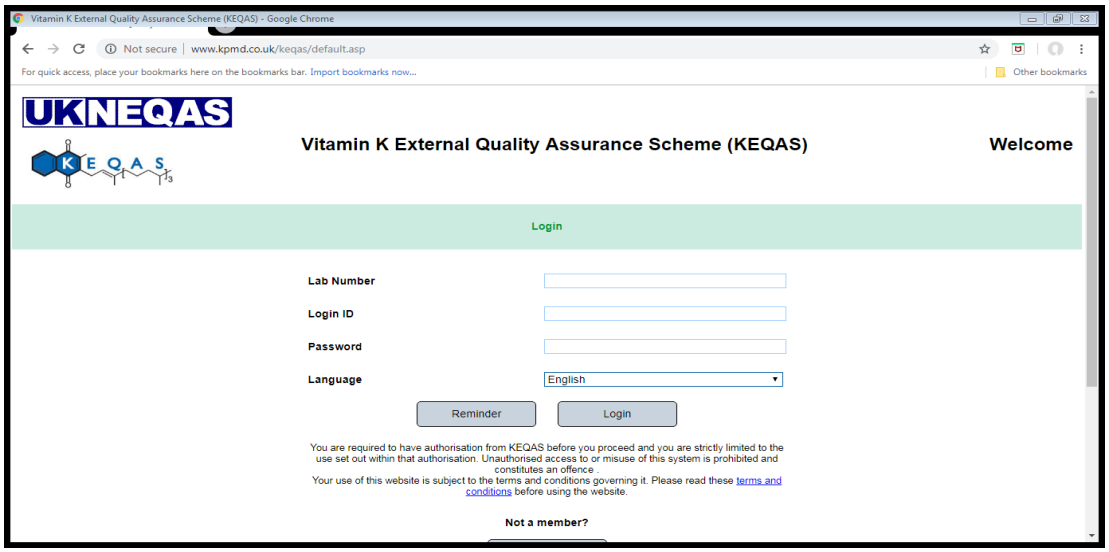
From January 2019 the method for submitting results and accessing reports for KEQAS changed to an online service: the EQA*lite* service for KEQAS can be accessed at www.kpmd.co.uk/keqas. All future results submissions should be made via this website to ensure your participation in the distribution rounds.

Please read this section on how to use the service before the next analysis period. If you have any queries please direct them to ella.freke@viapath.co.uk.

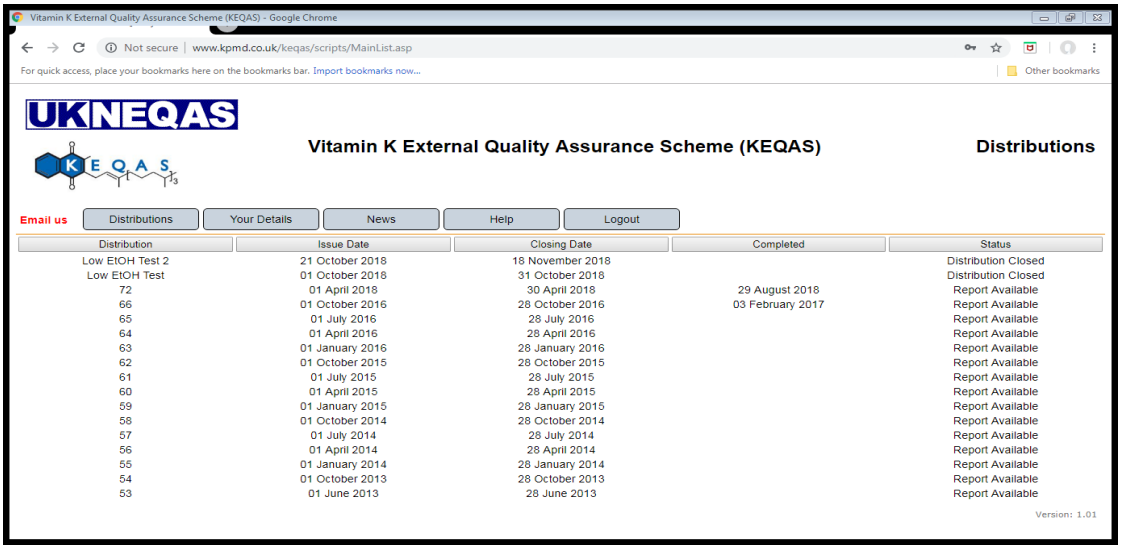
Please note using the back button on your browser may log you out. If you do need to go back to a different page, use the appropriate tabs on the website

Logging in

Go to www.kpmd.co.uk/keqas using Chrome (version 61) as your web browser. Log in to the EQA*lite* service using your KEQAS laboratory number, login ID (this is your email address that is registered with the scheme on enrolment) and password. If it is your first time using the website, your password will be emailed to you by clicking on 'Reminder'.

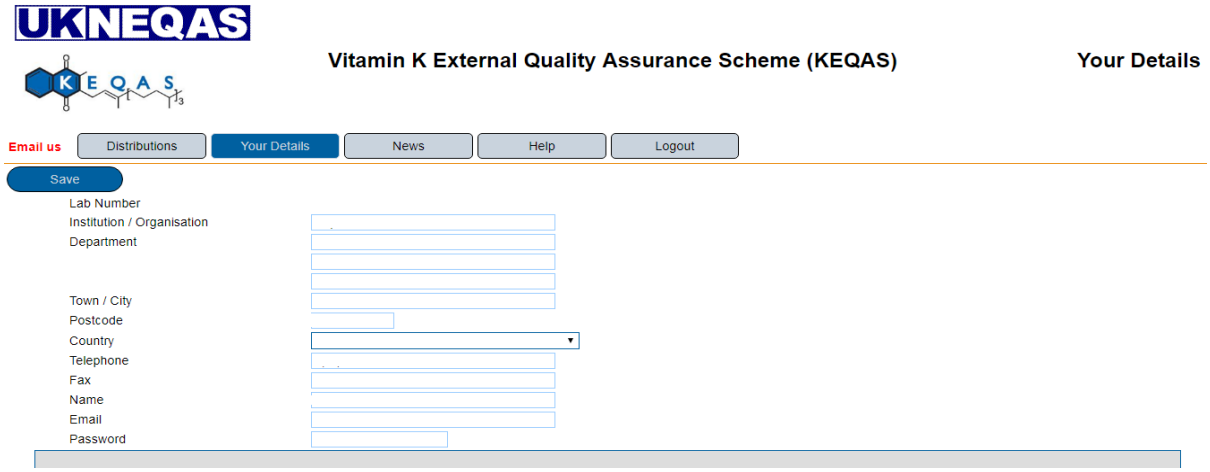


Your homepage will show your list of distributions:



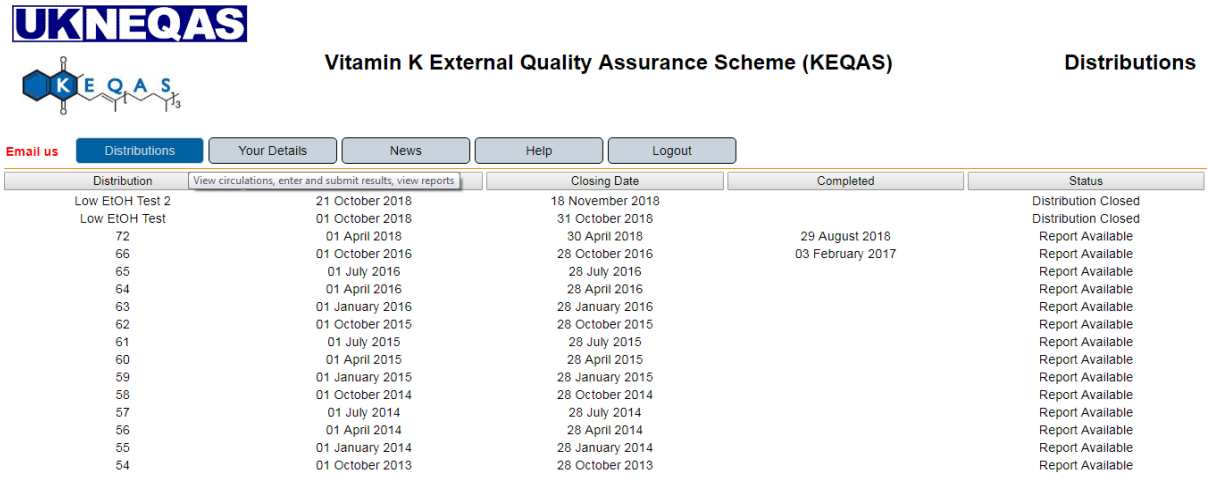
Your details

It is important that you check your details are correct. These can be viewed using the 'Your Details' tab. If any of the information is incorrect, please update and click 'Save':



Entering your results

On your homepage click on the 'Distributions' tab and click on the distribution for which you would like to submit your results:



UKNEQAS

KEQAS

Vitamin K External Quality Assurance Scheme (KEQAS) **Distributions**

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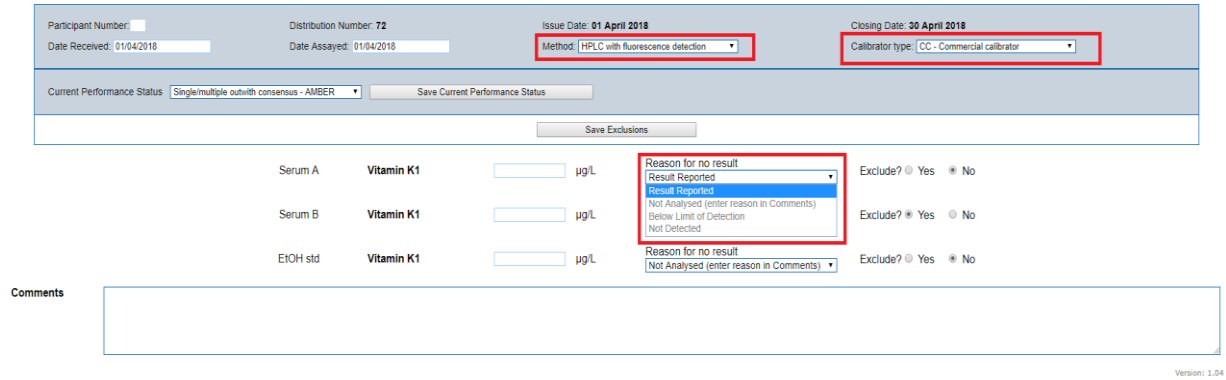
Distribution	View circulations, enter and submit results, view reports	Closing Date	Completed	Status
Low EtOH Test 2	21 October 2018	18 November 2018		Distribution Closed
Low EtOH Test	01 October 2018	31 October 2018		Distribution Closed
72	01 April 2018	30 April 2018	29 August 2018	Report Available
66	01 October 2016	28 October 2016	03 February 2017	Report Available
65	01 July 2016	28 July 2016		Report Available
64	01 April 2016	28 April 2016		Report Available
63	01 January 2016	28 January 2016		Report Available
62	01 October 2015	28 October 2015		Report Available
61	01 July 2015	28 July 2015		Report Available
60	01 April 2015	28 April 2015		Report Available
59	01 January 2015	28 January 2015		Report Available
58	01 October 2014	28 October 2014		Report Available
57	01 July 2014	28 July 2014		Report Available
56	01 April 2014	28 April 2014		Report Available
55	01 January 2014	28 January 2014		Report Available
54	01 October 2013	28 October 2013		Report Available

Enter the dates the samples were received and assayed and select the method and calibrator type used. Results must be reported in the standard units of µg/L. Results cannot be entered after the distribution has been closed. Amended reports are not yet available using this service.

If you were unable to report a result, please select from the drop-down menu next to 'Reason for no result'. If the sample was not analysed, please provide a reason for this in the 'Comments' field. If you are reporting a result, please select 'Result Reported':



Participant Number: | Distribution Number: **72** | Issue Date: **01 April 2018** | Closing Date: **30 April 2018**

Date Received: 01/04/2018 | Date Assayed: 01/04/2018 | Method: HPLC with fluorescence detection | Calibrator type: CC - Commercial calibrator

Current Performance Status: Single/multiple outwith consensus - AMBER | Save Current Performance Status

Save Exclusions

				Reason for no result	Exclude?
Serum A	**Vitamin K1**		µg/L	Result Reported	Yes / No
Serum B	**Vitamin K1**		µg/L	Result Reported / Not Analysed (enter reason in Comments) / Below Limit of Detection / Not Detected	Yes / No
EtOH std	**Vitamin K1**		µg/L	Not Analysed (enter reason in Comments)	Yes / No

Comments

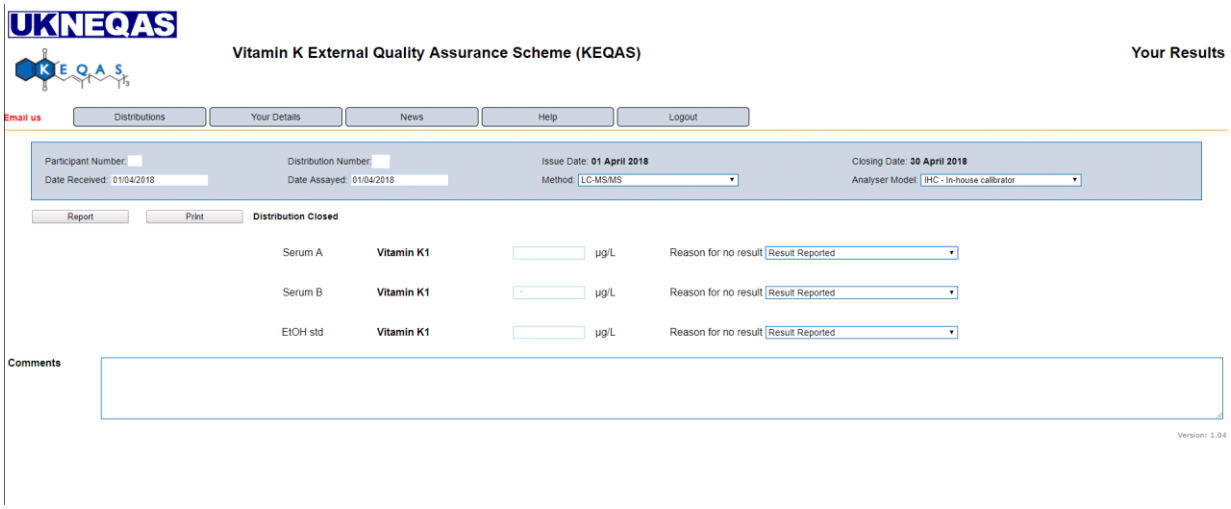
Version: 1.04

Once you have entered your results, a box will appear at the top of the page. Click 'Save' and then 'Submit'.

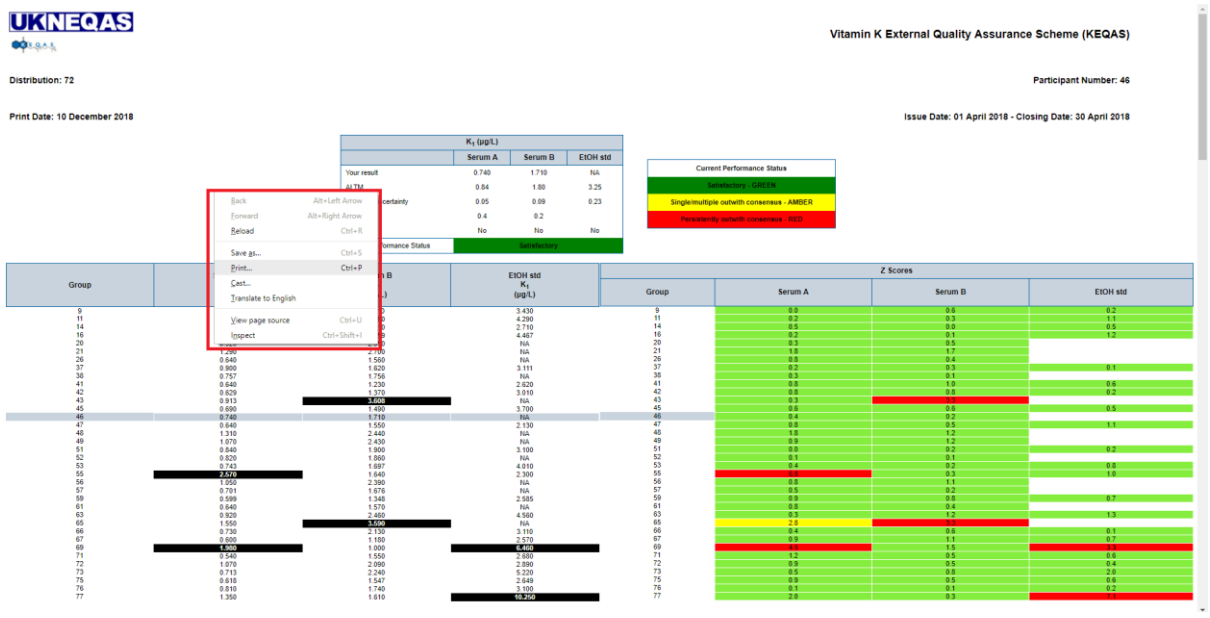
<u>Please note, you cannot change your results after you have clicked submit.</u>

Viewing your report

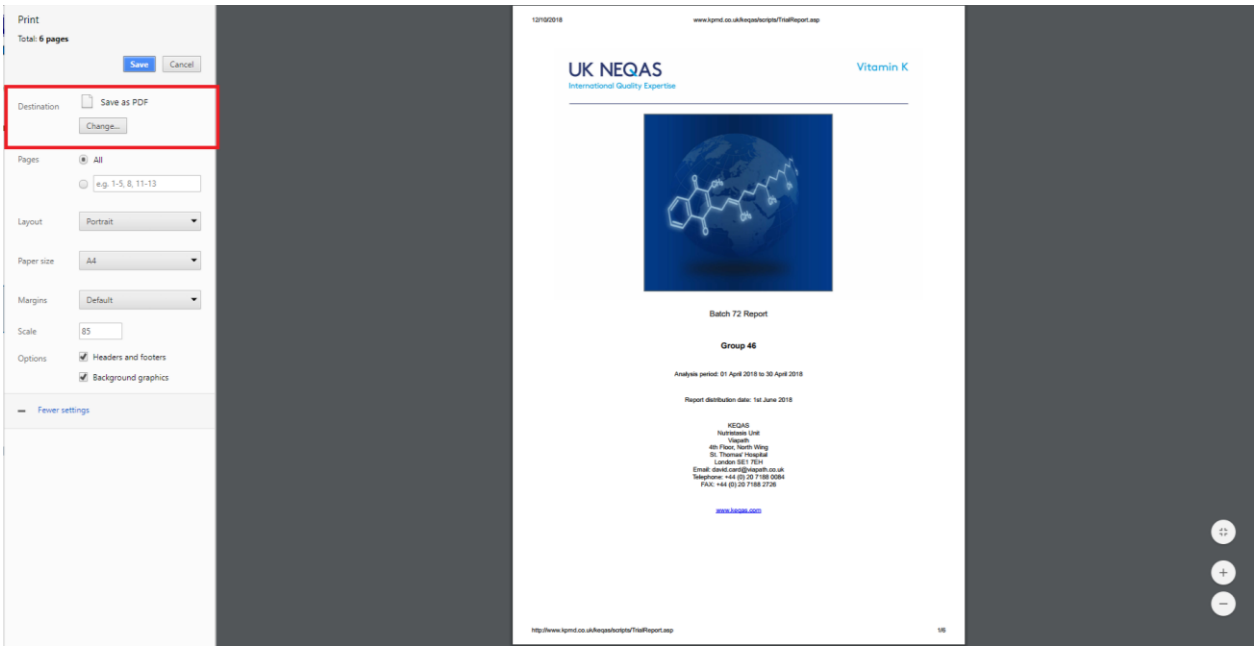
You will be notified via email when your report is available. To view your reports select the distribution number on the 'Distributions' page and click on 'Report':



To print your report, right-click and select 'Print Preview', as shown below. The scale can be adjusted to fit the report onto the page. Page numbers can be added by changing the settings on print preview in the browser. Participants who do not respond in a round can enter 'NA' (not analysed) using the drop-down menu, they will still be able to view their report even if they have not entered a result.



The report can also be saved as a PDF document by “printing” it to a PDF writer. On Chrome, a PDF can be created by changing the destination to ‘Save as PDF’:



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)
(iii) Not be identifiable as an obvious gross outlier

The ALTM represents the target concentration, Grubbs test identifies outliers that could distort the mean value for that sample. Obvious gross outliers not identified by Grubbs test may also be removed. The target for results is the current performance target (PT). The PT is calculated using data from serum samples excluding data from groups using commercial calibrators from the previous three years. It 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:

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

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 out with consensus result). Persistently out with consensus 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 out with consensus laboratories 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 • Current performance status • Z scores • Outliers' test
3	• Calibrator and method-specific means • Abbreviations and definitions
4	• Results plots
5	• Z scores plot • Standard deviation plot
6	• 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. Data from groups using commercial calibrators is excluded from calculation of the PT.

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*assigned value is < 0.3 x 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 Q-Pulse 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.

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 K_1) 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 K_1 (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 K_1 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, Harrington DJ. What's in a name? The pharmacy of vitamin K. *Br J Haematol*. 2016;174(6):989–990.

Card DJ, Gorska R, Harrington DJ. Laboratory assessment of vitamin K status. J Clin Pathol. 2020;73(2):70–75.

Card DJ, Hall A, Watson HG, Kitchen DP, Harrington DJ. Portion-controlled spinach for improved vitamin K antagonist anticoagulant control. *Br J Haematol*. 2020;10.