TESTQUAL
PROFICIENCY TESTING SCHEMES

TestQual, S.L.

(Proficiency Testing Schemes)

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TestQual 173 PROTOCOL

N-nitrosamines and N-nitrosable compounds in latex inflatable ballons

Summary of changes

Changes marked with blue or highlighted blue.

Rev01 →

·Acceptance deadline of applications updated.

·Text added regarding LOQs and applications acceptance.

·More info added about subcontracting done.

·If z'-score is issued, it will be accredited and the difference against z-score will be included in the report.

·Other minor changes

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1. INTRODUCTION

This document describes the **protocol** of the **TestQual 173** Proficiency Test (P.T.), which belongs to the analysis of **N-nitrosamines and N-nitrosable compounds** in **Latex inflatable balloons**.

TestQual, S.L. is committed to maintain the confidentiality of all laboratories from the beginning of the proficiency test, after it and at all times.

2. OBJECTIVE

The objective of the **TestQual 173** Proficiency Test is to evaluate the quality and accuracy of the results sent by the participating laboratories. Because of this, proficiency testing is an essential element of laboratory quality assurance. It helps to control and detect errors in their results or methods of analysis.

3. CALENDAR

The following table shows the schedule for this proficiency test:

Date	Activity	Carried out by
Week 42 (20/Oct/22)	***Deadline to reserve PT sample. Ensured reviewing* of application.***	Participants
Week 46 (16/Nov/22)	***Deadline to send application (acceptance depends on availability)***	***Participants***
Week 47 (21/Nov & 22/Nov/22)	Sample delivery	TestQual
Week 50 (16/Dec/22)	Final date to receive results	Participants
Week 52 (29/Dec/22)	Final report	TestQual

Participants are requested in the application to submit their LOQ/LOQs, for PTs with multiple possible analytes, if participants analyse above a certain percentage (as described in our internal procedure) of present/planned analytes the participation is accepted, a laboratory code is granted and sent by email to confirm the participation acceptance to the user of the TestQual's account.

The dates of this calendar might be slightly changed according to the development of the proficiency test during the year. However, any change would be notified to all participants announcing it on our website *www.TestQual.com*.

The **coordinator** of this proficiency test will be Jose Pedro Navarro. ***Vicente***. Any question regarding the development of the proficiency test, ***the application status or any other query*** can can be consulted by email to jpnavarro@testqual.com.

4. PARTICIPATION REQUEST AND WEBPAGE

TestQual webpage and all of its documents are available in both English and Spanish.

NEW CLIENT

If your laboratory has not participated before in one of our proficiency tests you will have to register on our website using the REGISTER form.
Once you have completed and sent the form you as soon as possible the website administrator will activate your account. If some more information is needed to complete your registration, one of the PT coordinators will get in contact with you through the contact data you introduced during your registration.
If you have any doubt or if we can help you with anything you can always contact any of our PT coordinators, you will find all the contact data in the Contact tab from our website.
For those laboratories that want to have more than one contact per account or wants more than one laboratory per account will have to contact one of the PT coordinators or through our website to be instructed how to proceed.

APPLICATION FOR THE PROFICIENCY TEST

To participate in this proficiency test the application is needed to be done through the website.
You can send your application by entering our website and going to the Proficiency tests tab , if you are interested in one of our PTs, by clicking on the name of the PT or the shopping cart you will enter a page with general information about that PT and there you can download the present document (protocol), at the bottom of the page will be a link to start the APPLICATION FORM, all inscriptions must be done before the scheduled date in the calendar.

Once the application has been sent, as soon as possible, it will be checked by the website administrator and you will receive an email with the participation code. This code will be just known by the organizer and the participating laboratory, and will be kept confidential at all times.

Just one application per exercise can be sent by each laboratory, being not allowed for a laboratory to participate with two different codes.

Laboratories with two or more laboratories in its network can participate with all of their laboratories in the same PT. Different applications are needed for each laboratory. In case of doubt you can contact the PT coordinator for guidance.

The applications of the laboratories will be studied and accepted in base of the quantification limits of the analytes of the P.T., making sure the participant will analyse and quantify correctly a certain minimum of analytes, ensuring that if you are given a laboratory code is because the participation will be useful and that minimum has been met or surpassed.

TestQual can anticipate that the number of participants of this P.T. will be around 11, being 11 the minimum participants for this proficiency test to take place.

5. PREPARATION. *DISTRIBUTION AND CONTROL*

TestQual 173 scheme is a proficiency test based in the analysis of **N-nitrosamines and N-nitrosable compounds** in **Latex inflatable balloons**. The material is bought to a specialised company in Murcia and analysed by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.
The material is frozen with liquid nitrogen and triturated to little pieces (0.3 cm^2 approximately or less), homogenised for some time at a controlled temperature and then will be packed in a special plastic bag that is thermally sealed in vacuum or cylindrical jar with pressure seal and screw cap. The samples are stored at -21ºC in controlled temperature freezers until distribution.

The distribution of samples was subcontracted to a courier previously homologated by TestQual.

The main criteria being the courier's delivery time to ensure the receival of the sample is correct in the participant's facilities.

In addition to this, TestQual stablished other characteristics important for a courier like shipping management (tracking, notifications, exceptions), and ensuring the delivery conditions are proper (low breakage/lost ratio, keeping of cold chain, required documentation), always checking and evaluating they are complying with TestQual's requirements.

For homogeneity assessment purpose, ten of the prepared samples are analysed in duplicate by TestQual´s collaborator laboratory under repeatability conditions.

For stability assessment purpose, three samples are analysed, in duplicate, before, during and at the end (once all laboratories have sent their results) of the proficiency test.

The quality controls subcontracted by TestQual, including verification of adequacy of the matrix, homogeneity/stability quality controls and any other analytical study required by TestQual will be subcontracted to an accredited laboratory in ISO/IEC 17025 into force.

In the evaluation report will be included the conclusions and if applies, any comment regarding homogeneity &/or stability. Additionally, the results of these tests and the spiking evaluation is available to all participants of this proficiency test upon request.

6. SAMPLE SHIPMENT

The shipment of the test materials will take place on the date shown in the calendar, to the address provided by each laboratory during the registration in TestQual page, the shipment address may be checked/changed by logging in the client area.

About **20 g** of test material will be sent by courier service (MRW, DHL, UPS, TNT, or Fedex depending on the destination and estimated transit). The material will be sent in padded envelope that ensures proper conditions of arrival of the sample. A second sample may be

requested within two days from the reception of the first one by any participant who finds the package or the sample damaged.

7. CONCENTRATION RANGES, SIGMA OBJECTIVE AND ANALYTES

In this proficiency test, the N-nitrosamines and N-nitrosable compounds will be found **between 20µg/Kg and 300 µg/Kg.**

The **sigma objective ($\hat{\sigma}$)** which works in this scheme will be the **25% of the assigned value for N-nitrosamines and 50% for N-nitrosable compounds**. These values were chosen taking into TestQual's experience in these tests.

The result of the laboratory should be expressed as µg/Kg of the following N-nitrosamines and N-nitrosable compounds that are detected and quantified:

N-nitrosodibenzylamine (NDBzA)

N-nitrosodibutylamine (NDBA)

N-nitrosodiethanolamine (NDELA)

N-Nitrosodiethylamine (NDEA)

N-nitrosodiisobutylamine (NDiBA)

N-Nitrosodiisopropylamine (NDiPA)

N-Nitrosodimethylamine (NDMA)

N-Nitrosodipropylamine (NDPA)

N-nitroso-N-ethyl-N-phenylamine (NEPhA)

N-Nitroso-N-methylethylamine (NMEA)

N-nitrosomorpholine (NMOR)

N-nitroso-N-methyl-N-phenylamine (NMPhA)

N-nitrosopiperidine (NPIP)

N-Nitrosopyrrolidine (NPYR)

8. RESULTS EXPRESSION

Each participant must analyse the sample received according to their routine procedures, and fill up the RESULTS form of its private area of the website www.testqual.com with only one value.

The results should be expressed in **µg/Kg**. The number of significant figures and the units are shown as they are sent by the laboratories.
The method used for the analysis should be sent when filling up the results form.

The organizer should get the results before the deadline of the scheme.

9. STATISTICAL EVALUATION

TestQual will develop the following statistical evaluation:

TestQual considers as an **extreme outlier** any data which differs more than **50 %** of the average of all results reported by the laboratories, according to the Harmonize Protocol of the IUPAC. These extreme values will not be included in the calculation of the assigned value.

Once received all the results, TestQual evaluates the unimodality of all the values with a histogram built with the Kernel densities, being explained in the final report which is the followed procedure in case there is more than one distribution.

The **assigned value (*X*)** is determined using the robust average of the results considered valid for statistical computing (after eliminating the extreme outliers), according to the standard ISO13528 up to date.

The **standard uncertainty (u_x)** is calculated using robust statistics from the following formula:

$$u_x = s^*/\sqrt{p}$$

Being s^* the robust standard deviation of the data and p the number of results considered.

The following condition must be fulfilled in order to discard the contribution of the uncertainty:

$$u_x \leq 0{,}3\ \hat{\sigma}$$

In case the inequation $u_x \leq 0{,}3\ \hat{\sigma}$ is not fulfilled, the participants of the scheme will be informed in the report that the uncertainty is not negligible. For the parameters/analytes in which this situation occurs, ***the following calculation will be made:***

$$z'\text{-score} = (x_i - X)/\sqrt{\hat{\sigma}^2 + U_x^2}$$

Where x_i is the value reported by the laboratories, X is the assigned value, $\hat{\sigma}$ is the target standard deviation for each analyte and Ux is the uncertainty of the assigned value.

The criteria for defining the z'-score values are:

	$\lvert z' \rvert$	≤2	*Satisfactory*
2 <	$\lvert z' \rvert$	≤3	*Questionable*
	$\lvert z' \rvert$	>3	*Unsatisfactory*

The z'-score is a subestimation of the z-score, for this reason, for those analytes in which the uncertainty of the assigned value cannot be neglected and a z'-score is issued, it will be accompanied by the percentual difference against z-score, this way participants should be able to complete evaluate their performance.

The evaluation could be informative if the difference between scores surpasses the limit contemplated in our procedure. If any analyte or evaluation is informative it will be indicated in the report through marking and a legend.

The **standard deviation for proficiency assessment**, also named **target standard deviation, ($\hat{\sigma}$),** comes from this formula:

$$\hat{\sigma} = b_i \cdot X$$

Being $b_i = \%_{DSRA} / 100$, and $\%_{DSRA}$ is the assigned relative standard deviation.

In this case, the assigned relative standard deviation is **25 % for N- nitrosamines and 50% for N-nitrosable compounds**. This value is fixed previously by the organizer and explained in the section seven of this protocol.

Proficiency assessment (z-score): This parameter shows the competence and accuracy of the laboratory. It is calculated using the following formula:

$$z = (x_i - X)/ \hat{\sigma}$$

Where x_i is the value reported by the laboratories, X is the assigned value, and $\hat{\sigma}$ is the target standard deviation for each analyte.
The criteria for defining the z-score values are:

	$\|z\|$	≤ 2	*Satisfactory*
$2 <$	$\|z\|$	≤ 3	*Questionable*
	$\|z\|$	> 3	*Unsatisfactory*

False negatives: Any analyte not reported in the results that were in the sample above the limit of quantification previously established to the proficiency test established by the organization **(20 µg/Kg)**. TestQual assigns to all false negatives a result equal to half the laboratory limit of quantitation (LOQ/2).

False positives: Those analytes reported in the results, which were not present in the test material, are reported by the participant at concentrations higher than the limit of quantification of the P.T. **(20 µg/Kg).**

Other results: Those analytes reported in the results, above the limit of quantification of the P.T. **(20 µg/Kg)**, these results cannot be classified as false positives and therefore are included in the report as "other results.

Testing for sufficient homogeneity:
Once the samples from both lots of samples were prepared, ten of them were chosen at random and sent to be analysed by TestQual's collaborator laboratory. Once received the results, a statistical evaluation was performed, according to the IUPAC Harmonic Protocol.

The acceptance criterion to ensure that the randomly chosen samples were homogeneous was that the square of the estimated sampling standard deviation is below the critical value for accepting proper homogeneity:

$$S_{sam}^2 < c$$

In the first place to check the criterion, S_{sam}^2 which is the estimated sampling standard deviation, is calculated from:

$$S_{sam} = (\frac{Vs}{2} - S_{an})$$

Firstly *Vs* is the variance of the sums S_i:

$$Vs = \sum \frac{(S_i - \bar{S})^2}{m-1}$$

Where S_i can be obtained from the addition of each duplicate result from the homogeneity; $\bar{S}$ is the mean of all S_i and *m* is the number of samples (10 samples).

And secondly S_{an}^2 , which is the experimental estimate of analytical standard deviation, is obtained following formula:

$$S_{an}^2 = \frac{\sum D_i}{2m}$$

where D_i is the result of the subtraction of each pair of replicates from the homogeneity and *m* is the number of samples.

Lastly to calculate the critical value *c* was obtained from:

$$c = F_1 \cdot \sigma_{all}^2 + F_2 \cdot S_{an}^2$$

Being F_1 and F_2 constants with values equal to 1.88 and 1.01 respectively for 10 samples. S_{an}^2 has already been calculated and σ_{all}^2 is obtained from:

$$\sigma_{all}^2 = (0.3 \cdot \hat{\sigma})^2$$

where $\hat{\sigma}$ is the target standard deviation, which is calculated with the formula:

$\hat{\sigma}$ = **0,25** $\cdot \bar{X}$ for N-nitrosamines.

$\hat{\sigma}$ = **0,50** $\cdot \bar{X}$ for N-nitrosable compounds.

Being $\bar{X}$, the mean of the 20 values from the homogeneity.

<u>Testing for sufficient stability:</u>

Three samples will be analysed, in duplicate, before, during and at the end (once all laboratories have sent the results) of the proficiency test. The acceptance criteria to ensure the samples have been stable during the whole P.T. are the following:

$$|(X_{t1} - X_{t2})/ X_{t1}| \cdot 100 \leq 10\%$$
$$|(X_{t1} - X_{t3})/ X_{t1}| \cdot 100 \leq 10\%$$

Being $|(X_{t1} - X_{tn})/ X_{t1}|$ the difference between the average of the samples analysed before, during and at the end of the proficiency test.

10. EVALUATION REPORT

Once received and statistically evaluated all of the participating laboratories results, TestQual will send a final report that summarizes the participation of each laboratory.
This final report will be sent to the laboratories via e-mail in PDF format, and it can also be downloaded from the client area of each participant on www.testqual.com. Laboratories may request TestQual to send them the reports in paper by mail as well.
If any participant wants to appeal against the assessment program performance, a written appellation must be sent by email to jpnavarro@testqual.com, explaining their reasons for it.

11. REFERENCES

TestQual Proficiency Testing Schemes are based on the following standards:

UNE-EN ISO/IEC 17043 into force. Conformity assessment- General requirements for proficiency testing.

ISO13528 into force, second edition 2015-08-01. Statistical methods for use in proficiency testing by interlaboratory comparison.

THE INTERNATIONAL HARMONIZED PROTOCOL FOR THE PROFICIENCY TESTING OF ANALYTICAL CHEMISTRY LABORATORIES