



TESTQUAL

PROFICIENCY TESTING SCHEMES

TestQual, S.L.

(Proficiency Testing Schemes)

Pol. Industrial Oeste

Av. Principal, Parcela 21/1

CP 30169, San Ginés, Murcia

Telephone: 868 949 486 / 676 367 555

PROFICIENCY TEST

TestQual 22D PROTOCOL

***NITROSAMINES INCLUDING NDMA &
NDEA in DIOVAN (ACTIVE INGREDIENT
VALSARTAN, FROM SARTAN DRUG
FAMILY)***

CANCELLED

Summary of changes

Changes marked with *blue* or **highlighted blue**.

Rev01 →

·Calendar updated

Rev02 → **CANCELLED**

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0. GLOSARY AND ABBREVIATIONS

Text	Abbreviation
TestQual	TQ
Proficiency test	PT / P.T.
Limit Of Quantification	LOQ
Not Analysed	NA

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 22D** Proficiency Test (P.T.), belonging to the analysis of **nitrosamines including NDMA & NDEA in DIOVAN (ACTIVE INGREDIENT VALSARTAN)**

In the present document is detailed how to start working with TestQual, send your application to participate, the statistic that will be applied and information about the evaluation report.

TestQual, S.L. is committed to maintaining confidentiality with the information of each laboratory from the beginning of the proficiency test.

2. OBJECTIVE

The objective of the **TestQual 22D** Proficiency Test is to evaluate the quality of the results sent by the participating laboratories. Because of this, proficiency testing is an essential element of laboratory quality assurance. It can help to control and detect errors in their results or methods of analysis.

3. CALENDAR

The following table shows the program for this proficiency test:

Date	Activity	Carried out by
CANCELLED	Deadline to reserve samples	Participants
CANCELLED	Final date to submit applications	Participants
CANCELLED	Sample delivery	TestQual
CANCELLED	Final date to submit results	Participants
CANCELLED	Evaluation report (Email and/or client area)	TestQual

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. Any question regarding the development of the proficiency test can be consulted by email to jpnavarro@testqual.com.

4. REGISTER AND PARTICIPATION REQUEST (APPLICATION FORM)

NEW CLIENT

If your laboratory has not participated before in one of our proficiency tests you will have to register on the [REGISTER](#) form.

Once you have completed and sent the form you will have to wait until the activation of the account from the website administrator. If some more information is needed someone from our team will get in contact with you through the phone or email you used during your registration.

You can find our contact data at the end of this protocol.

For those laboratories that require more than one contact per account or are in a situation not contemplated in this protocol will have to contact the organizer using the Contact tab to be instructed how to proceed.

APPLICATION FOR THE PROFICIENCY TEST

To participate in this proficiency test or be updated via email of any changes regarding this proficiency test is required to have a laboratory code. To get your laboratory code is needed to apply through the website or have it arranged by the coordinator at least 24h before the shipping of the samples.

In the Proficiency Tests Tab on our website, you will have to select the proficiency test you want to participate, by clicking its name or the shopping cart you will enter the page with general information and a summary of that proficiency test, there you can find the present document (the protocol) and the button to start the application.

If you did not log in before you will be requested to do so and then the website will require you to submit your Limit Of Quantification (LOQ) for the Nitrosamines including NDEA & NDMA's you will study. The compounds left as NA (NOT ANALYSED) will NOT appear in the Results form and therefore will not be able to send results for those Nitrosamines including NDEA & NDMA's through the website.

Once the application has been sent, as soon as possible, it will be checked by the website administrator, and you will be sent an email with the participation code. This code will be just known only by the organizer and the participant and will be always kept confidential, even after the proficiency test finishes.

You can check on the dashboard of your client area if an application you sent has been accepted or is still pending.

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

The applications of the laboratories will be studied and accepted in base of the quantification limits of the analytes of the proficiency test and if the logistics allow the sample shipping without risk of deterioration.

TestQual can anticipate that the number of participants of this proficiency test will be 11, being that same number the minimum number of participants for the proficiency test to take place. In some extraordinary cases the PT might be distributed with less participants, that being the case TestQual would notify beforehand shipping the samples to each participant.

5. PREPARATION AND QUALITY CONTROLS

TestQual 22D scheme is a proficiency test based in the analysis of **nitrosamines including NDMA & NDEA in DIOVAN (ACTIVE INGREDIENT VALSARTAN)**, the pills will be frozen and smashed until an homogeneous powder is obtained. The powder, while being kept at cryogenic temperatures will be spiked with a number of nitrosamines and NDMA & NDEA, the powdered matrix will be mixed, frozen and re-mixed at temperature-controlled environment to ensure homogeneous distribution and maximum stability of the analytes.

Samples will be stored in a freezer (-25°C) until further distribution.

Before samples are distributed, random samples will be selected to be analysed in duplicate to assess the homogeneity of the lot, likewise, random samples will be reserved to be analysed in duplicate at different times for stability assessment purpose.

6. TEST MATERIAL AND 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 in the application. Specific delivery dates can change from the scheduled dates of the calendar, but all changes will be announced both in the website and/or by email to the registered laboratories.

This PT will consist in a single round in which will be sent a sample of approximately **1 g of test material**. The samples will be sent by courier service (MRW, FedEx, DHL or TNT, depending on the destination).

The material will be sent with materials and package that ensures the samples arrives correctly. The transit will be 1, 2 or 3 days to the destination country, depending on the location of the receiving laboratory.

The shipping conditions for this PT are:

Room temperature (below 30°C at all times, as recipe recommends), with enough padding and an padded envelope to ensure the sample arrives without leakage or breaking.

The shipping costs are not included in the price displayed on the website, which can only be seen if you are registered and logged in. To get an approximation of the shipping costs you can get your quotation by using the contact data at the end of this protocol.

If the package and/or the sample arrived damaged, defective, or not valid the participating laboratory will have to notify of this to the coordinator before two working days to get another sample, if no remaining samples would be available if the participant made any payment in advance TestQual will return the money or reach another agreement with the participant.

Before the shipment, TestQual will send the instructions for storage and analysis via email and confirm the distribution date. You can request a paper copy to be attached to the package and/or TestQual might decide to include it in addition to have it sent by email.

From TestQual we encourage our participants to read it carefully and follow its instructions, as it can help to correctly conserve the sample and increase the reproducibility of the analysis.

You can request a digital copy of this document by letting us know through any communication channel.

7. CONCENTRATION RANGES AND RESULTS EXPRESSION

For this proficiency tests the **range of concentration** for the target analytes might be between **50 and 500 µg/Kg**.

Some nitrosamines of the following group will be present:

N-nitrosodibenzylamine (NDBzA)

N-Nitrosodipropylamine (NDPA)

N-nitrosodibutylamine (NDBA)

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

N-nitrosodiethanolamine (NDELA)

N-Nitroso-N-methylethylamine (NMEA)

N-Nitrosodiethylamine (NDEA) (presence Guaranteed)

N-nitrosomorpholine (NMOR)

N-nitrosodiisobutylamine (NDiBA)

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

N-Nitrosodiisopropylamine (NDiPA)

N-nitrosopiperidine (NPIP)

N-Nitrosodimethylamine (NDMA) (presence Guaranteed)

N-Nitrosopyrrolidine (NPYR)

Each participant laboratory must analyse the sample received according to their routine procedure and fill up the RESULTS form of its client are of the website www.TestQual.com with just one value per analyte.

The results should be expressed in **µg/Kg**. The number of significant figures and the units are to be chosen by laboratories and will be displayed in the report as received through the website.

The method used for the analysis of each compound informed should be sent when filling up the results form.

The organizer should get the results before the fixed data of the scheme.

If you have any problem submitting your results you can contact the coordinator of the PT for guidance or help.

8. 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 by Kernel test, 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 ISO 13528 into force.

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:

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

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 %**.

This value is fixed previously by the organizer based in the experience of TestQual organizing this and similar proficiency tests.

Proficiency assessment (z-score): This Nitrosamines including NDEA & NDMA 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

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 Nitrosamines including NDEA & NDMA's/analytes in which this situation occurs, the evaluation will be issued as z'-score according to the following calculation:

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

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 U_x is the uncertainty of the assigned value.

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 is in the sample above the limit of quantification previously established for this proficiency test by the organization and above the LOQ of the participant laboratory (**10 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 is not present in the test material, and is reported by the participant at concentrations higher than the limit of quantification of the P.T. (**10 µg/Kg**).

Testing for sufficient homogeneity:

Once the samples are prepared ten of them will be chosen at random and sent to be analysed by TestQual's collaborator laboratory. Once received the results, a statistical evaluation will be performed, according to the IUPAC Harmonic Protocol.

The acceptance criterion to ensure that the randomly chosen samples are homogeneous is 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, was calculated from:

$$S_{sam} = \left(\frac{V_S}{2} - S_{an} \right)$$

Firstly V_S is the variance of the sums S_i :

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

Where S_i was 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 the next 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.

In second place to check the criterion for sufficient homogeneity 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:

$$\hat{\sigma} = 0.25 \cdot \bar{X}$$

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

Testing for sufficient stability:

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 proficiency test are the following:

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

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.

9. EVALUATION REPORT

Once received and statistically evaluated all the participating laboratories' results, TestQual will send a final report that summarizes the participation of each laboratory.

This final report will be received by the laboratories via e-mail in PDF format or an email notifying that the report is now available to be downloaded from the private area of each participant in.

If desired, the laboratory may request the report in paper, and it will be sent to its laboratory by mail.

If a participant wishes to appeal against the assessment program performance, a written appellation must be sent by e-mail to jpnavarro@testqual.com explaining the reasons for it.

10. CONTACT

TestQual puts at your disposal any of the following means to contact our team:

Website:	Contact
Email:	jpnavarro@testqual.com
Office phone:	+34 868 94 94 86
Mobile phone:	+34 676 367 555

11. REFERENCES

TestQual Proficiency Testing Schemes are based on the following standards:

UNE-EN ISO/IEC 17043, first edition 2010-02-01. Conformity assessment- General requirements for proficiency testing.

ISO13528:2015, 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

END OF DOCUMENT

