TestQual, S.L.

(Proficiency Testing Schemes)

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TestQual 157 PROTOCOL

Food additives: preservatives in olive

Summary of changes

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

Rev01➔PT code was wrong, changed from "21B and 21C" to 157

Rev02➔ ·Calendar updated (wrong dates were displayed).

·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

Rev03➔ updated sample size

Rev04➔ updated calendar

Rev05➔ updated calendar

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0. GLOSARY AND ABREVIATIONS

Text	Abbreviation
TestQual	TQ
Proficiency test	PT / P.T.
Limit Of Quantification	LOQ
NA	Not Analysed
ML	Maximum Level (according to EU regulation)

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 157** Proficiency Test, belonging to the analysis of **food additives (Food preservatives)** in **Olive**.

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 keep the confidentiality of all the information provided from the beginning of the proficiency test and at all times, even after it has finished.

2. OBJECTIVE

The objective of the **TestQual 157** 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 will help to control and detect errors in their results or methods of analysis.

3. CALENDAR

The following table shows the program for this proficiency scheme (for both samples):

Date	Activity	Carried out by
-	Deadline to reserve PT sample. Ensured reviewing* of application.	Participants
Week 23 (15/Jun/22)	Deadline to send application (acceptance depends on availability)	Participants
Week 24 (20 & 21 /Jun/22)	Sample delivery	TestQual
Week 26 (08/Jul/22)	Final date to submit results	Participants
Week 28 (15/Jul/22)	Final report (Email and/or client area)	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 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 parameters 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 that parameters 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 kept confidential at all times, even after the proficiency test finishes.

To receive a sample from both matrixes you will have to send the application for both proficiency tests on our website.

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 around 11-15, being 11 the minimum participants for the proficiency test to take place.

5. PREPARATION. DISTRIBUTION AND CONTROL

TestQual 157 scheme is a proficiency test based in the analysis of **food additives (Food preservatives)** in **Olive** that can be found within this matrix. The material will be bought in a specialized shop in Murcia, Spain and analysed by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.
The material will be homogenizer properly and once the lot of samples is ready they will be stored in a temperature-controlled freezer below -20ºC until the dispatch of the samples.

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.
Before the samples are distributed, for the assessment of the homogeneity of the lot of samples prepared, ten samples from the lot will be selected randomly and analysed in duplicate by TestQual´s collaborator laboratory under repeatability conditions. If the mean concentration obtained from this study is not within the planned range, the participants will be informed and a new distribution day might be scheduled if another spiking is deemed as necessary.

For stability assessment purpose, three samples are analysed, in duplicate, before, during and at the end (once all laboratories have sent the 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. TEST MATERIAL AND SAMPLE SHIPMENT

The shipment of the test materials (both matrixes) will take place on the date shown in the calendar, to the address provided by each laboratory in their client area. 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 mail to the inscribed laboratories.

This PT will consist in a single round in which will be sent a sample of approximately **20-50 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 in a way that ensures proper arrival conditions to the laboratory.

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.

A second test material for both matrixes can be requested if necessary. 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.

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 conserve correctly 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, SIGMA OBJECTIVE AND ANALYTES

The range of concentration for the target analytes of this proficiency test might by between **10 µg/kg** and **the ML in these matrixes** approximately**.**

The **sigma objective ($\hat{\sigma}$)** which works in this scheme will be the **one detailed in section 9 of this protocol**. This value has been chosen according to the experience of similar proficiency tests organized by TestQual.

The possible food additives (Food preservatives) in the Olive are from the list below:

Possible additives (preservatives)	Expressed as	Reporting units

Possible additives (preservatives)	Expressed as	Reporting units
E202 potassium sorbate E203 calcium sorbate	SORBATE	µg/Kg
E210 B*enzoic Acid* E211 Sodium Benzoate E212 Potassium Benzoate E213 Calcium Benzoate	BENZOATE	µg/Kg

8. RESULTS EXPRESSION

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/parameter.

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 logging in to your client area or submitting your results you can contact the coordinator of the PT for guidance or help.

Once the results are sent you can check if they were correctly recorded by accessing the detailed information of this proficiency test, which can be accessed in your client area.

9. STATISTICAL EVALUATION

TestQual will develop the following statistical evaluation for each matrix of this Proficiency scheme:

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 and evaluate the performance of the laboratory with z-score:

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

In case this condition 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:

$$|z'| \quad \leq 2 \qquad \text{Satisfactory}$$
$$2 < \quad |z'| \quad \leq 3 \qquad \text{Questionable}$$
$$|z'| \quad > 3 \qquad \text{Unsatisfactory}$$

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:

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

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

In this case, the assigned relative standard deviation will be calculated with the Horwitz-Thomson mathematical model for the target standard deviation. Expecting it to be between 10 and 20 **%.**

The assigned value will be converted to non-dimensional units (C, in g/g units), and according to the following range of application the target standard deviation will be calculated with the applicable equation:

Range of application	Equation
C < $1,2 \cdot 10^{-7}$	$\hat{\sigma}_{PT} = 0.22 \cdot C$
$1,2 \cdot 10^{-7} \leq C \leq 0,138$	$\hat{\sigma}_{PT} = 0.22 \cdot C^{0.8495}$
C > 0,138	$\hat{\sigma}_{PT} = 0.1 \cdot C^{0.5}$

Where $\hat{\sigma}_{PT}$ is the target standard deviation for each analyte.

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

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

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:

$$
\begin{array}{lccl}
 & |z| & \leq 2 & \text{Satisfactory} \\
2 < & |z| & \leq 3 & \text{Questionable} \\
 & |z| & > 3 & \text{Unsatisfactory}
\end{array}
$$

False negatives: Any analyte not reported in the results that is in the sample above the limit of quantification previously established to the proficiency test established by the organization **(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:

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

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

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

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:

$$\hat{\sigma} = 0.15 \cdot \bar{X}$$

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:

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

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 received by the laboratories via e-mail in PDF format, but also can be downloaded from the private area of each participant in www.TestQual.com.
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.

11. 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

12. REFERENCES

TestQual Proficiency Testing Schemes are based on the following standards:
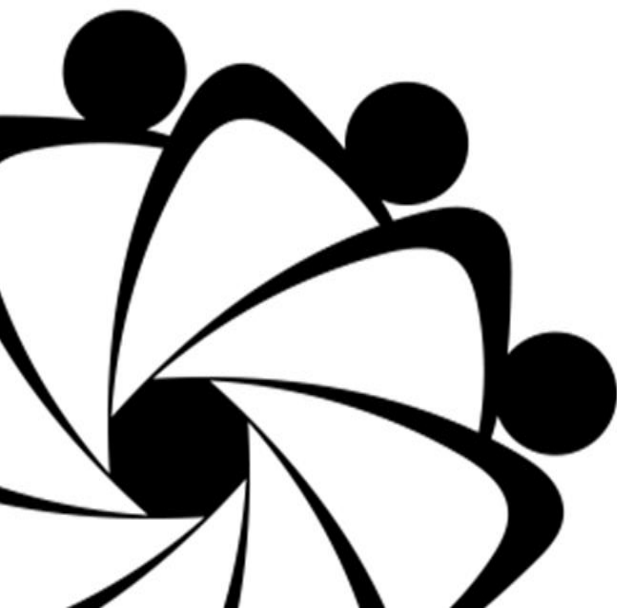
UNE-EN ISO/IEC 17043, first edition 2010-02-01. Conformity assessment- General requirements for proficiency testing.

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

Council Directive 2001/111/EC of 20 December 2001 relating to certain sugars intended for human consumption.

he European Parliament and of the Council of 16 December risation procedure for food additives, food enzymes and food

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