



# **TESTQUAL**

## **PROFICIENCY TESTING SCHEMES**

**TestQual, S.L.**

**(Proficiency Testing Schemes)**

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# **TestQual 165 PROTOCOL**

## ***Additives: Colorants in Saffron***

## 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

Rev02 →calendar, amount of sample, statistical design and appellation time for the evaluation report updated.

Rev03 →calendar updated

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## 0. GLOSSARY AND ABBREVIATIONS

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

## 1. INTRODUCTION

This document describes the **protocol** of the **TestQual 165** Proficiency Test (P.T.), belonging to the analysis of **Additives: Colorants** in **Saffron**.

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 165** 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 test:

Date	Activity	Carried out by
<i>Week 38 (20/Sep/22)</i>	Deadline to reserve PT sample. Ensured reviewing* of application.	Participants
<i>Week 39 (28/Sep/22)</i>	Deadline to send application (acceptance depends on availability)	Participants
<i>Week 40 (03/Oct &amp; 04/Oct/22)</i>	Sample delivery	TestQual

<a href="#">Week 43 (28/Oct/22)</a>	Final date to submit results	Participants
<a href="#">Week 45 (11/Nov/22)</a>	Final report (Email and/or client area)	TestQual

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\*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](http://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](mailto: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 those 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 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.

According to the experience, TestQual can anticipate that the number of participants of this proficiency test will be around **7-15**, being 11 the minimum participants for the proficiency test to take place.

## 5. PREPARATION. DISTRIBUTION AND CONTROL

**TestQual 165** scheme is a proficiency test based in the analysis of **Additives: Colorants in Saffron** that has been spiked with **standards**.

The material will be bought in an specialized shop in Spain and analysed by a subcontracted laboratory that holds the standard ISO/IEC 17025 into force.

The material is grounded and spiked with a solution with the analytes of the proficiency test and poured into a homogenizer with controlled temperature to ensure complete homogeneity. Once the lot of samples is ready, samples in vacuum-themosealed bags will be stored in a temperature-controlled freezer below -20°C 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 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 by mail to the registered laboratories.

This PT will consist in a single round in which will be sent a sample of approximately **15 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, The material will be sent in a vacuum packed, heat sealed plastic bag in dark conditions (opaque and padded envelope).

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 can be requested date 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 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, SIGMA OBJECTIVE AND ANALYTES

For this proficiency tests the range of concentration for the target analytes might be between **200** and **1000 µg/Kg approximately**. This range is not absolute and depending on the various factors some or no analyte might be within this range. This range is given to provide participants a sense of the concentration ranges we usually work and expect in these kind of proficiency tests (combination of analyte/matrix).

The maximum concentration can vary depending on the MRL of the analytes in the matrix.

The **sigma objective ( $\hat{\sigma}$ )** which works in this scheme can be checked on section 9 of this protocol.

Amaranth	Amaranto
Orange II	Orange II
Quinoline Yellow	Amarillo 2G
Tartrazine	Amarillo anaranjado
Roceline	Amarillo de Quinoleina
Azorubine	Rojo ponceau 4R
Ponceau 4R red	Tartrazina
Yellow-orange	Rocelina
Yellow 2G	Azorrubina

## 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](http://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 DESIGN

Any participant in this proficiency test can request further information or support about the statistical design that TestQual has developed.

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.

If enough valid results are provided and/or the uncertainty is negligible 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.

If the number of participants or the number of valid results does not reach a certain number and the uncertainty obtained using robust averages is not negligible, both the assigned value and the uncertainty will be calculated as the spiking/characterization done (design based on ISO 13528 into force).

This is done to evaluate the results against an independent value from the participants' results, since using a low number of results might cause a bias and to not detect action signals if required. The assigned value obtained from robust statistic is always compared to the assigned value obtained from characterization of the spiking done and depending on the situation TestQual might decide to use either of the assigned value, justifying that decision on the evaluation report.

If the consensus assigned value is not used, then the characterization of the spiking and the uncertainty will be calculated based in a suitable model and according to ISO 13528 into force.

The standard uncertainty ( $u_x$ ) when the assigned value is obtained from the consensus it 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.

When the assigned value is obtained from the spiking done, the uncertainty is calculated accounting all sources of uncertainty according to our internal procedure and according to ISO 13528 into force.

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 obtained from the HORWITZ-THOMSON calculation.



Depending on the concentration of the assigned value the following equations will be applied:

Equations:

$$\sigma_{\text{Hor}}=0.22 \cdot C \text{ if } C < 1.2 \cdot 10^{-7}$$

$$\sigma_{\text{Hor}}=0.02 \cdot C^{0.8495} \text{ if } 1.2 \cdot 10^{-7} \leq C \leq 0.138$$

$$\sigma_{\text{Hor}}=0.01 \cdot C^{0.5} \text{ if } C < 0.138$$

Being  $\sigma_{\text{Hor}}$  the estimated standard deviation from the equations from Horwitz-Thompson.

After calculating the Horwitz-Thompson standard deviation it will be compared with the robust standard uncertainty obtained from the robust analysis of the results to ensure the estimated RSD is acceptable.

To check if the obtained standard deviation is acceptable the Horwitz Ratio is calculated:

$$\text{HorRat} = s^* / \sigma_{\text{Hor}}$$

Where  $s^*$  is the robust standard deviation and

Since the results obtained are from different laboratories, reproducibility conditions apply, therefore, to check if the ratio and the estimated relative standard deviation is acceptable the following range is applied:

If HorRat is within 0.5 – 2 → Acceptable and  $\sigma_{\text{Hor}}$  is used when calculating the scores of the participants. If HorRat is not within that range the evaluation and score of participants will be obtained using the robust standard deviation.

**Proficiency assessment (z-score):** This score 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 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  $U_x$  is the uncertainty of the assigned value.

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

	$ z'  \leq 2$	Satisfactory
2 <	$ z'  \leq 3$	Questionable
	$ z'  > 3$	Unsatisfactory

The  $z'$ -score is a underestimation 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.

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 (**200 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. (**200 µg/Kg**).

Other results: Analytes that were not present in the blank item/ are not spiked that are reported below the minimum concentration of the PT (200µg/Kg) that cannot be classified as false positives. If enough results are provided these analytes would be evaluated.

**Testing for sufficient homogeneity:**

Once the samples are prepared between four and ten of them will be chosen at random and sent to be analysed by TestQual's collaborator laboratory in duplicate in repeatability conditions (eight to twenty results will be generated). The number of samples analyzed will be chosen based on:

TestQual's experience homogenizing this or similar matrixes, the analyzed number of samples is representative to the number of samples prepared, as well as other factors that might affect this proficiency test taking place. Once received the results, a statistical evaluation will be performed, with a modified calculation from the Harmonic Protocol published by IUPAC and based in ISO 13528 into force.

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 obtained in function of the values from the significance tables: Chi square distribution and F distribution, both with 95% confidence. For example: equal to 1.88 and 1.01 respectively for 10 samples, while it would be 2.1 and 1.43 respectively for 7 samples. The less samples analysed the higher  $c$  will be, making harder the criteria to consider the lot homogeneous.

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

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.

If  $S_{sam}^2 < c$  is true, then the lot prepared will be considered sufficiently homogeneous and only then it would be distributed. If the results from the homogeneity test does not meet the criteria TestQual would communicate any change required in the proficiency test (new lot will be prepared, new calendar, etc.).

**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 or an email notifying that the report is now available to be downloaded from the private area of each participant in [www.TestQual.com](http://www.TestQual.com).

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

In the event that a participant wishes to appeal against the assessment program performance, a written appellation must be sent by e-mail to [jpnavarro@testqual.com](mailto:jpnavarro@testqual.com) explaining the reasons for it.

30 days will be available after issuing the final report to inform of any mistake or to appeal against the evaluation, after that time and once all appellations have been reviewed, those that were justified, a new revision of the evaluation report would be issued. This does not impede that new revisions might be issued if deemed necessary, always being communicated to the participants if a new revision is available.

## 11. CONTACT

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

Website:	<a href="#">Contact</a>
Email:	<a href="mailto:jpnavarro@testqual.com">jpnavarro@testqual.com</a>
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.

Thompson, M.; Ellison, S. L. R.; Wood, R. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories: (IUPAC Technical Report). *Pure and Applied Chemistry* **2006**, 78 (1), 145–196.

Rivera, C.; Rodríguez, R.; Pimentel, S. C. HORWITZ EQUATION AS QUALITY BENCHMARK IN ISO / IEC 17025 TESTING LABORATORY. **2011**.

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