TestQual, S.L.

(Proficiency Testing Schemes)

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TestQual 115 PROTOCOL

Ochratoxin A and Aflatoxins in paprika

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 115** Proficiency Test (P.T.), which belongs to the analysis of **Ochratoxin A and Aflatoxins B, G and totals** in **Paprika**.

TestQual, S.L. is committed to maintain the confidentiality of all of each laboratory from the beginning of the proficiency test.

2. OBJECTIVE

The objective of the **TestQual 115** 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
18/Oct/19	Final date to receive applications	Participants
22/Oct/19	Sample delivery	TestQual
15/Nov/19	Final date to receive results	Participants
Week 48-49	Final report	TestQual

The dates of this calendar might slightly vary according to the development of the proficiency tests. However, any modification in the dates will be announced in advance on our website *www.testqual.com*.

The **coordinators** of this proficiency test will be Jose Pedro Navarro and María Ángeles Garrido. Any question regarding the development of the P.T. can be consulted by email jpnavarro@testqual.com or magarrido@testqual.com

4. PARTICIPATION REQUEST (SUBSCRIPTION FORM)

NEW CLIENT

If your laboratory has not participated before in one of our proficiency tests you will have to fill in the REGISTER form.

Once you have completed and sent the form as soon as possible the page administrator will activate your account and notify you when it has been enabled. If some more information is needed our team will get in contact with you through the phone or email to complete your registration.

In case of urgency because of a deadline or if you have a doubt you can contact our team through the Contact tab from our website and ask for a quote or for help.

For those laboratories that require more than one contact per account or that works with more than one laboratories at the same time will have to contact us using the Contact tab or by email to be instructed how to proceed.

APPLICATION FOR THE PROFICIENCY TEST

To participate in this proficiency test is needed to apply through the website.
In the Proficiency Tests Tab on our website will have to be selected the P.T. you want to participate, by clicking the shopping cart or the name of the PT you will enter the page with general information regarding that proficiency test, the present document (the protocol) and 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 send the application, 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 by the organizer and the 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.

The applications of the laboratories will be studied and accepted in base of the quantification limits of the analytes of the P.T. and its geographical location if needed, so the logistics allow the sample shipping without risk of deterioration.

According to the experience, TestQual can anticipate that the number of participants of this P.T. will be around 20, being 11 the minimum participants for this proficiency test.

5. TEST MATERIAL

TestQual 115 scheme is a proficiency test based in the analysis of **Ochratoxin A and Aflatoxins** in **Paprika**. The material is bought to a specialized company in Murcia and analysed by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.
The material is poured into a homogenizer to ensure complete homogeneity and vacuum packed, then is stored in a dark, cold and dry place.

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 test material might be sent in a single sample of paprika with both Ochratoxin A and Aflatoxins or in two separate paprika samples. In any case, the samples would be sent at the same time as scheduled in the calendar, the shipment will be done to ensure that no crossed contamination can occur and with the samples clearly labelled with the analytes that can be expected in each sample.

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 (which can be changed in the Client Area). Specific delivery dates may change from the scheduled dates of the calendar, but any change will be announced both on the website and by email to the participants.

About **130 g** of test material will be sent by courier service (MRW, DHL or TNT, depending on the destination).The material will be sent in a padded and opaque envelope that ensures the temperature conditions of the package during the entire shipment. The transit will be 1, 2 or 3 days, depending on the location of the receiving laboratory.
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, **Ochratoxin A and some of the Aflatoxins** will be in a concentration higher than **0,1 µg/Kg.**
The **sigma objective ($\hat{\sigma}$)** which works in this scheme will be the **30% of the assigned value for Ochratoxin A** and **22% for Aflatoxins B, G and total aflatoxins,** according to the Horwitz equation, modified for concentrations lower than $1{,}2*10^{-7}$. These values were chosen based on the legislation No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 (…).

The result of the laboratory should be express as the content of the following:

Ochratoxin A, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Total Aflatoxins

8. RESULTS EXPRESSION

Each participant must analyse the sample/s 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. **Results must be corrected regarding the recovery.**

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 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 ISO13528 up to date.

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

$$U_x = 1{,}25 \cdot (s^*/\sqrt{p})$$

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 this condition is not fulfilled, the participants of the scheme will be informed, and the uncertainty will have to be taking into account for the assigned value assessment.

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 **30 % *for Ochratoxin A and 22% for aflatoxins***. This value is fixed previously by the organizer based in the experience of TestQual organizing similar proficiency tests.

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 for this proficiency test established by the organization **(0.1 µg/Kg for Ochratoxin A. 0,2 µg/kg for Aflatoxin B1 and G1. 0,06 µg/Kg for Aflatoxin B2 and G2)**. 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, and are reported by the participant at concentrations higher than the limit of quantification of the P.T. **(0.1 µg/Kg for Ochratoxin A. 0,2 µg/kg for Aflatoxin B1 and G1. 0,06 µg/Kg for Aflatoxin B2 and G2)).**

Testing for sufficient homogeneity:

Once the 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, was 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 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:

$$\hat{\sigma} = \mathbf{0.3} \cdot \bar{X} \text{ (Ochratoxin A)}$$

$$\hat{\sigma} = \mathbf{0.25} \cdot \bar{X} \text{ (Aflatoxins)}$$

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

Testing for sufficient stability:

Three samples were analysed, in duplicate, before, during and at the end (once all laboratories have sent the results) of the proficiency test. With these values, a study is performed to ensure the stability of the analytes. 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 sent to the laboratories via e-mail in PDF format, and it can also be downloaded from the private 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 mail to jpnavarro@testqual.com or magarrido@testqual.com, explaining their reasons for it.

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

COMMISSION REGULATION (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis (Text with EEA relevance)
Official Journal L 147, 17.5.2014, p. 29–43