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

ENAC
INTERCOMPARACIONES
Nº 9/PPI014

The parameters/matrixes marked with (*) are not accredited

CANCELLED

TestQual 139 A+B PROTOCOL

Multimycotoxin in Paprika and Corn flour (*)

0. GLOSARY AND ABREVIATIONS

Text	Abbreviation
TestQual	TQ
Proficiency test	PT
Limit Of Quantification	LOQ
NA	Not Analysed
OTA	Ochratoxin A
DON	Deoxynivalenol
ZON	Zearalenone

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 139A+ B** Proficiency Test (P.T.), which belongs to the analysis of **Ochratoxin A, Aflatoxins B, G, Deoxyvalenol (*), Fumonisin B1(*) and B2(*), HT-2 (*)and H-2 Toxin(*) and Zearalenone(*) in Paprika and Corn flour (*).**

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 maintain the confidentiality of all of each laboratory from the beginning of the proficiency test.

2. OBJECTIVE

The objective of the **TestQual 139A+B** 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
CANCELLED	Final date to submit applications	Participants
	Sample delivery	TestQual
	Final date to submit results	Participants
	Final 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. PARTICIPATION REQUEST (SUBSCRIPTION 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 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.

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

5. TEST MATERIAL

TestQual 139A+B scheme is a proficiency test based in the analysis of **various mycotoxins** in **Paprika and Corn flour (*).** The material is bought to a specialized company in Spain and analysed by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.
The matrix can be naturally positive, negative or spiked for some or all of the following analytes:

Aflatoxin B1	**Aflatoxin B2**	**Aflatoxin G1**	**Aflatoxin G2**	**Total aflatoxins**	**Fumonisin B1**(*)
Fumonisin B2(*)	**Deoxyvalenol**(*)	**HT-2 Toxin**(*)	**H-2 Toxin**(*)	**Zearalenone**(*)	**OTA**

The material is poured into a homogenizer to ensure complete homogeneity and vacuum packed, and then is stored in a dark, cold and dry place.

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 their results) of the proficiency test.

The test material might be sent in a single sample or in two separate Paprika and Corn flour (*) 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 has to be studied 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 **100 g** of test material will be sent by courier service (MRW, Fedex, 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.

The shipping costs are not included in the price displayed on the website. To get an approximation you can get your quotation by using the contact data at the end of this protocol.

A second test material can be requested if the participating laboratory justify, within two days from the reception of the sample, that the received package or the sample is damaged.

Along with the shipment, TestQual includes a document with extra instructions for the storage and analysis. 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 you can find below, in this protocol.

7. CONCENTRATION RANGES, SIGMA OBJECTIVE AND ANALYTES

In this proficiency test, at least 2 of the mycotoxins from the table will be above the concentration displayed.

The **sigma objective or target standard deviation ($\hat{\sigma}_p$)** which works in this scheme can be found in the table below.
The maximum value for the $\hat{\sigma}_p$ is chosen from the legislation No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 (…) .

Analyte	Minimum Concentration (µg/kg)	Maximum concentration (µg/kg)	$\hat{\sigma}_{tar}$ (%)
Ochratoxin A	**0.1**	**<3**	**30**
Aflatoxin B1	**0.2**	**<5**	**22**
Aflatoxin B2	**0.06**	**<120**	**22**
Aflatoxin G1	**0.2**	**<120**	**22**
Aflatoxin G2	**0.06**	**<120**	**22**
Deoxyvalenol(*)	**3**	**<750**	**40**
Fumonisin B1(*)	**15**	**<1000**	**60**
Fumonisin B2(*)	**15**	**<1000**	**60**
HT-2 Toxin(*)	**4**	**<50**	**50**
H-2 Toxin(*)	**4**	**<50**	**50**
Zearalenone(*)	**5**	**<75**	**50**

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:

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

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 as shown in table from section 7 of this protocol. This value is fixed previously by the organizer based in the experience of TestQual organizing similar proficiency tests, the applicable legislation and its experience.
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 **(see table below)**. 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. **(see table below)**.

Analyte	Concentration (µg/kg)
OTA	**0.1**
Aflatoxin B1	**0.2**
Aflatoxin B2	**0.06**
Aflatoxin G1	**0.2**
Aflatoxin G2	**0.06**
Deoxyvalenol(*)	**3**
Fumonisin B1(*)	**15**
Fumonisin B2(*)	**15**
HT-2 Toxin(*)	**4**
H-2 Toxin(*)	**4**
Zearalenone(*)	**5**

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:

$$\hat{\sigma} = (\hat{\sigma}_{tar}/100) \cdot \bar{X}$$

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, 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

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