



**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*



**TestQual 130 PROTOCOL**  
***Pesticides Residues in Wheat flour***

## 1. INTRODUCTION

This document describes the **protocol** of the **TestQual 130** Proficiency Test (P.T.), belonging to the analysis of **pesticides** in **Wheat flour**.

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 130** 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
<b>22/May/20 (Week 21)</b>	Final date to receive applications	Participants
<b>26/May/20 (Week 22)</b>	Sample delivery	TestQual
<b>18/Jun/20 (Week 25)</b>	Final date to receive results	Participants
26/Jun/20 (Week 26)	Final report	TestQual

The dates of this calendar can slightly change according to the development of the P.T. during the year. However, any modification in the dates will be announced in advance on our website [www.testqual.com](http://www.testqual.com).

The **coordinator** of this proficiency test will be Jose Pedro Navarro. Any question regarding the development of the PT 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.

In case of urgency or if you have a doubt you can contact our team through the Contact tab from our website.

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 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 it 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.

During the application you will have to enter your Limit Of Quantification (LOQ) for the pesticides you will study. Those compounds that are left as NA (NOT ANALYSED) will NOT appear in the Results form and therefore will not be able to send results through the form.

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, so the logistics allow the sample shipping without risk of deterioration.

According to the experience, TestQual can anticipate that the number of participants for this P.T. will be between **18** and **20** laboratories, being 11 the minimum participants of any proficiency test.

## 5. TEST MATERIAL

**TestQual 130** scheme is a proficiency test based in the analysis of **pesticide residues** in **Wheat flour** that has been treated with pesticide **standards**. The material has been bought in an ecological and specialised shop in Murcia and analysed by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.

The material will homogenised and then spiked with a solution with the analytes of the proficiency test. After complete homogenization it will be packed and properly stored until further shipment to participants

For **homogeneity** assessment purpose, ten of the prepared samples of the flour are analysed in duplicate by TestQual 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 the results) of the proficiency test.

## 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 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.

About **100 g** of **wheat flour** will be sent by courier service (MRW, DHL or TNT, depending on the destination) at the same time. The materials will be sent in a vacuum packed, heat sealed plastic bag in dark conditions.

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, any of the analytes to inform are in a concentration higher than **10 µg/Kg**. The **sigma objective ( $\hat{\sigma}$ )** which works in this scheme will be the **25 % of the assigned value for all the pesticides**. This value has been chosen according to the experience of TestQual organizing these proficiency tests.

The possible **pesticides** in the Wheat flour are from the list below:

2-Phenylphenol	Aldrin	Bromophos-ethyl
3-Hydroxy-carbofuran	Anthraquinone	Bromopropylate
Abamectin	Atrazine	Bupirimate
Acephate	Azinphos-methyl	Captan
Acetamiprid	Azoxystrobin	Carbaryl
Acetochlor	Benzoate Emamectin B1	Carbendazim
Aclonifen	Bifenthrin	Carbofuran
Acrinathrin	Boscalid	Chlorfenvinphos

Chlorfensón	Fluquinconazole	Oxydemeton-methyl
Chlorpyrifos	Flusilazole	Oxyfluorfen
Chlorpyrifos Methyl	Flutriafol	Paclobutrazol
Cyprodinil	Fonofos	Parathion
Cyfluthrin	Formothion	Parathion-methyl
Cypermethrin	Phosalone	Penconazole
Cyproconazole	Phosphamidon	Pendimethalin
Clothianidin	Phosmet	Permethrin
Deltamethrin	HCH-Alpha	Piperonyl butoxide
Demeton-S-methyl sulfone	(alpha-hexachlorocyclohexane)	Pyrimethanil
Diazinon	HCH-Beta	Pyraclostrobin
Difenoconazole	(Beta - hexachlorocyclohexane)	Pirimicarb
Diflubenzuron	HCH-Delta	Pirimicarb-desmethyl
Dimethoate	(Delta - hexachlorocyclohexane)	Prochloraz
Dimethomorph	HCH-Gamma	Procymidone
Dimoxystrobin	(lindane)	Propiconazole
Disulfoton	Heptachlor	Quinoxifene
Endosulfan-alpha	Hexachlorobenzene	Quintozene
Endosulfan-beta	Hexaconazole	Spinosad A+D
Endosulfan-sulfate	Hexaflumuron	Tebuconazole
Endrin	Hexythiazox	Tebufenocid
Epoxiconazole	Imazalil	Tebufenpirad
Spiroxamine	Imidacloprid	Terbufos
Etaconazole	Iprodione	Thiabendazole
Ethion	Isofenphos-methyl	Thiacloprid
Ethoprophos	Isoproturon	Thiamethoxam
Etoxazole	Lambda-Cyhalotrina	Thiodicarb
Etrimfos	Linuron	Tiobencarb
Fenazaquin	Malaoxon	Tolclofos-methyl
Fenbuconazole	Malathion	Triadimefon
Fenhexamid	Methacrifos	Triadimenol
Fenitrothion	Methamidophos	Triazophos
Fenthion	Methomyl	Trifloxystrobin
Fenvalerate	Myclobutanil	Trifluralin
Fipronil	Omethoate	Vinclozolin
Fludioxonil		

## 8. RESULTS EXPRESSION

Each participant laboratory must analyse the sample received according to their routine procedure, and fill up the RESULTS form of its private are of the website [www.testqual.com](http://www.testqual.com) with just one value for each parameter detected.

The results should be expressed in  $\mu\text{g}/\text{Kg}$ . The number of significant figures and the units are shown as they were sent by the laboratories.

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 previously shown deadline for the test.

## 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 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:

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

In case this condition is not fulfilled, the participants of the scheme will be informed in the report, 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 **25 %** for all the **pesticides**. This value is fixed previously by the organizer based in the experience of TestQual organizing these and 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  \leq 2$	Satisfactory
$2 <$	$ z  \leq 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 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 from the prepared lot and will be 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  $\sigma_{all}^2$  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 from the wheat flour lot lot 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 P.T. 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.

## **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](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 [jnavarro@testqual.com](mailto:jnavarro@testqual.com) explaining the reasons for it.



## 11. CONTACT

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

Website:	<a href="#">Contact tab</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.

THE INTERNATIONAL HARMONIZED PROTOCOL FOR THE PROFICIENCY TESTING OF ANALYTICAL CHEMISTRY LABORATORIES

SANTE/12682/2019, 1st January 2020 Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.