TESTQUAL
PROFICIENCY TESTING SCHEMES

TestQual, S.L.

(Proficiency Testing Schemes)

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ENAC
INTERCOMPARACIONES
Nº 9/PPI014

Parameters marked with (*) are not accredited

TestQual 147 PROTOCOL

Fosetyl, phosphonic acid, Maleic hydrazide, ethephon (*) and glyphosate (*) in Arugula

0. GLOSARY AND ABREVIATIONS

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

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 147** Proficiency Test (P.T.), belonging to the analysis of **fosetyl, phosphonic acid, maleic hydrazide, *ethephon (*) and glyphosate (*)*** in **Arugula**.

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 147** 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
21/May/2021 (Week 20)	Final date to submit applications	Participants
15/Jun/2021 (Week 24)	Sample delivery	TestQual
09/Jul/2021 (Week 27)	Final date to submit results	Participants
30/Jul/2021 (Week 30)	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. 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.

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 24-30, being 11 the minimum participants for the proficiency test to take place.

5. TEST MATERIAL

TestQual 147 scheme is a proficiency test based in the analysis of **fosetyl, phosphonic acid, maleic hydrazide, *ethephon (*) and glyphosate (*)*** in **Arugula**. The material has been bought in an ecological shop in Spain and analysed by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force.

The material is cut in very small pieces, dropped into liquid N_2, once it is fully frozen, it is grounded into a fine powder, puree or juice, which will be 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, they will be stored in a temperature-controlled freezer below -20ºC until the dispatch of the samples.

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.

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 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 **100-150 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 insulated box that ensure the temperature conditions of the package during the whole shipment. The transit will be 1, 2 or 3 days to the destination country, depending on the location of the receiving laboratory. These boxes will be provided with either dry ice or cold packs (-21ºC) to keep the temperature for the transit.

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 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

In this proficiency test, any of the analytes to inform are in a concentration higher than **10 µg/Kg.**

The range of concentration for the target analytes (except sum) of this proficiency test might by between **10 and 300 µg/kg** approximately.

The **sigma objective ($\hat{\sigma}$)** which works in this scheme will be the **25% of the assigned value**. This value has been chosen according to the experience of similar proficiency test.

Each participant shall report his results as the European legislation 2016/75 (…) amending Annex III from No 396/2005 (…) levels for fosetyl (…):

- "Fosetyl_Al (Sum)" will be evaluated as "Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)".
- "Phosphonic Acid" as "Phosphonic acid and their salts".
- "Fosetyl" as only "Fosetyl".

The results for Maleic hydrazide will have to be reported as the EU Legislation No 777/2013 amending Annexes II, III and V from No 396/2005 (…) regards (…) Maleic hydrazide (…):

- "Maleic hydrazide" as "Maleic hydrazide".

The results for Ethephon will have to be reported in the results form EU applicable legislation 2017/1777 of 29 September 2017 amending Annexes II, III and IV to Regulation (EC) No 396/2005 (..), Ethephon, (..)

- "Ethephon" as "Ethephon"

The results for Glyphosate will have to be reported as EU applicable legislation No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 (..), Glyphosate (..)

- "Glyphosate" as "Glyphosate"

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.
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.
If the website do not requests your results for ethephon and glyphosate but you have analysed them, please, report your results through the "Notes" section in the results form in our website or contact us through email (contact data below).

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:

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 **25 %**. 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:

	$\lvert z \rvert$	≤ 2	Satisfactory
$2 <$	$\lvert z \rvert$	≤ 3	Questionable
	$\lvert z \rvert$	> 3	Unsatisfactory

False negatives: Any analyte not reported in the results that were 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 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. **(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 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:

$$\hat{\sigma} = 0.25 \cdot \bar{X}$$

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. With these values, a study is performed according the up to date guide (SANTE/12682/2019 *Guidance document on analytical quality control*), to analysis under repeatability conditions. 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 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.

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 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.

ISO 13528:2015, 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.

Commission Regulation (EU) 2016/75 of 21 January 2016 amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for fosetyl in or on certain products
OJ L 16, 23.1.2016, p. 8–20

Commission Regulation (EU) No 777/2013 of 12 August 2013 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for clodinafop, clomazone, diuron, ethalfluralin, ioxynil, iprovalicarb, **maleic hydrazide**, mepanipyrim, metconazole, prosulfocarb and tepraloxydim in or on certain products
OJ L 221, 17.8.2013, p. 1–48

Commission Regulation (EU) No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for emamectin benzoate, etofenprox, etoxazole, flutriafol, glyphosate, phosmet, pyraclostrobin, spinosad and spirotetramat in or on certain products
DO L 96 de 5.4.2013, p. 1/30

Commission Regulation (EU) 2017/1777 of 29 September 2017 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for Bacillus amyloliquefaciens strain FZB24, Bacillus amyloliquefaciens strain MBI 600, clayed charcoal, dichlorprop-P, ethephon, etridiazole, flonicamid, fluazifop-P, hydrogen peroxide, metaldehyde, penconazole, spinetoram, tau-fluvalinate and Urtica spp. in or on certain products
DO L 253 de 30.9.2017, p. 1/31