TestQual, S.L.

(Proficiency Testing Schemes)

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ENAC
INTERCOMPARACIONES
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Parameters marked with an asterisk (*) are not accredited by ENAC

TestQual 135 PROTOCOL

Fosetyl, Phosphonic Acid, Maleic hydrazide (*) and Ethephon (*) in Grape

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 135** Proficiency Test (P.T.), belonging to the analysis of **fosetyl, phosphonic acid, Maleic hydrazide (*) and ethephon (*) in Grape**.

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 135** 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
30/Oct/20 (week 44)	Final date to receive applications	Participants
17/Nov/20 (Week 47)	Sample delivery	TestQual
04/Dec/20 (Week 49)	Final date to receive results	Participants
18/Dec/20 (Week 51)	Final report	TestQual

The dates of this calendar may change slightly 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*.

The **coordinator** of this proficiency test will be Jose Pedro Navarro. Any question regarding the development of the P.T. 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.
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 of this P.T. will be between 15 to 20, being 11 the minimum participants of any proficiency test.

5. TEST MATERIAL

TestQual 135 scheme is a proficiency test based in the analysis of **fosetyl, phosphonic acid, Maleic hydrazide (*) and ethephon (*) in Grape**. The material has been bought in an ecological shop in Murcia 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, spiked with a solution with the analytes of the P.T., and dropped into liquid N_2. Once fully frozen, ground into a fine powder, which will be poured into a homogenizer to ensure complete homogeneity.

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 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 **200 g** of test material will be sent by courier service (MRW, 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, depending on the location of the receiving laboratory. These boxes will be provided with either dry ice or cold packs to keep the temperature.

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.

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 ".

EU Legislation No 399/2015 amending Annexes II, III and V from No 396/2005 (…) regards (…) ethephon (*) (…):

- "Ethephon " as "Ethephon "

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 with just 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.
The methods 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.

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 SANTE guide (SANTE/12682/2019 *Guidance document on analytical quality control*), referred 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 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 tab
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 (Text with EEA relevance)
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 Text with EEA relevance
OJ L 221, 17.8.2013, p. 1–48

COMMISSION REGULATION (EU) 2015/399 of 25 February 2015 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 1,4-dimethylnaphthalene, benfuracarb, carbofuran, carbosulfan, ethephon , fenamidone, fenvalerate, fenhexamid, furathiocarb, imazapyr, malathion, picoxystrobin, spirotetramat, tepraloxydim and trifloxystrobin in or on certain products.
Text with EEA relevance
OJ L 71, 14.3.2015, p. 1–55