



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 55

PROTOCOL TestQual 178

Multi-analysis *in Avocado*

- ***Multiresidues pesticides***
- ***Fosetyl, phosphonic acid***
- ***Chlorate and perchlorate***
- ***Glyphosate***

SUMMARY OF CHANGES

Changes are marked in electric blue or **blue highlighted white**.

Rev00 → rev01:

- Calendar updated
- Number of participants updated (currently expected: 12-15)
- Comment about storage and shipping improved (frozen).
- Comment included about blank items belonging to a different lot than the PT samples.
- Improved comment about conditions to calculate assigned value.
- Summarized homogeneity checks and interpretation of results.
- Updated bibliography reference to ISO 13528:2022

Rev01 → rev02:

- Updated calendar. Distribution from 3rd July to 5th July.
- Updated amount of sample from 150 to 120g.

Rev02 → rev03:

List of possible multiresidues pesticides residues is increased.

Increased number of participants expected.

How to submit results updated.

Modified calculation of the false negatives when no LOQ is available.

Rev04 →

- Increased deadline to submit results until 2023/07/28
- Report issued the same week than the date from previous revision of this protocol.

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0. GLOSARY AND ABBREVIATIONS

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

1. INTRODUCTION

This document describes the **protocol** of the **TestQual 178** Proficiency Test (P.T.) in **Avocado**, for the analysis of the **participant's choice** from the following group of **analytes**:

Group of analytes	Name
1	Multiresidues pesticides,
2	Fosetil, phosphonic acid
3	Chlorate and perchlorate
4	Glyphosate

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 178** 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 (YYYY/mm/dd)	Activity	Carried out by
-	Deadline to reserve PT sample.	Participants
-	Deadline to request participation*	Participants
-	Sample delivery	TestQual
2023/07/28	Final date to submit results	Participants

*Acceptance of the participation between the deadline to reserve the PT sample and the deadline to request the participation will depend on availability of the proficiency test.

Participants are requested in the application to submit their LOQ/LOQs. For PTs with multiple groups of analytes, for each group that the participant has submitted LOQ/LOQs, if a certain percentage (described in our internal procedure) of the present/planned analytes is analysed, then the participation is accepted, and a laboratory code is generated and sent by email to confirm the participation acceptance to the user of the TestQual's account.

The dates of this calendar and the definitive shipping distribution might be changed depending on the development of the proficiency test. The definitive distribution date is confirmed when the sample instructions are sent, which, besides the instructions for the sample, contains the deadline to submit the results (which might update and override the here displayed deadline).

The sample instructions are exclusively sent to inscribed participants. These possible changes would be notified to all participants through our website www.TestQual.com and/or by email.

The **coordinator** of this proficiency test will be Jose Pedro Navarro. Vicente. Any question regarding the development of the proficiency test, the application status or any other query 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 using 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. 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 to be updated via email of any changes regarding this this, it 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 the latest, on the deadline to request the participation.

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 image you will enter the proficiency test page, which contains 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.

You will be required to log in to start the application and then the website will require you to submit your Limit Of Quantification (LOQ) for the parameters you will study. **The analytes left as NA (NOT ANALYSED) will NOT appear in the Results form** and therefore will not be able to send results for those parameters through the website.

Once the participation has been requested, 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 13-15, being 11 the minimum participants for the proficiency test to take place.

5. TEST MATERIAL. CONTROL AND DISTRIBUTION.

TestQual **178** scheme is a proficiency test based in the analysis of Multiresidues pesticides, Fosetil, phosphonic acid, Chlorate and perchlorate in Avocado that has been spiked or were present in the matrix.

The material will be bought in a specialized shop in Spain and verified as adequate to prepare the samples by a subcontracted laboratory that holds the standard UNE-EN ISO/IEC 17025 into force. Blank items will be reserved to be acquired along the proficiency test, the blank item can be shipped along the PT sample, saving the shipping costs. The blank items will be available at a reduced cost for the participants of this PT. Blank samples are guaranteed to have a concentration <FP/FN (concentration limit to determine False Negatives and/or False positives) for the analytes of your interest (according to the LOQ you submitted and intended analytes to be analysed).

The subcontracted laboratory will analyse randomly selected sample to ensure the homogeneity and during and after the proficiency test more samples to check the stability of the parameters through the duration of the round.

Blank items belong to a different lot than the prepared PT samples.

Once the matrix is deemed adequate the material is cut in very small pieces, dropped into liquid N2, 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 -20°C approximately until the dispatch of the samples.

As previously mentioned, before the samples are distributed, the homogeneity of the lot will be assessed analysing in repeatability conditions randomly selected samples in duplicate. 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, a total of three samples will be analysed, in duplicate, before, during and at the end (once all laboratories have sent the results) of the proficiency test.

The quality controls subcontracted by TestQual, including verification of adequacy of the matrix, homogeneity/stability quality controls and any other analytical study required by TestQual will be subcontracted to an accredited laboratory in ISO/IEC 17025 into force.

In the evaluation report will be included the conclusions and if applies, any comment regarding homogeneity &/or stability. Additionally, the results of these tests and the spiking evaluation is available to all participants of this proficiency test upon request.

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. The participant is responsible to communicating TestQual if the address is changed or update the delivery address in their client area at least 2 working days before the shipping.

*Specific delivery dates can be changed from the scheduled dates of the calendar, but all changes will be announced either in the website or by email to the registered laboratories. Before shipping the samples TestQual will email the registered laboratories the sample instructions and will confirm the definitive distribution date, deadline to submit results and date of the report, if necessary, the dates shown in the sample instructions override the calendar here shown and the new dates will be updated in the PT page.

This PT will consist in a single round in which will be sent a sample of approximately **120 g** of test material. The samples will be sent by courier service (MRW, FedEx, DHL or TNT, depending on the destination). The material will be sent with materials and package that ensures the samples arrives correctly. The transit will be 1, 2 or 3 days to the destination country, depending on the location of the receiving laboratory.

The shipping conditions for this PT are:

FROZEN, isothermal boxes will be used and filled with either dry ice or cold packs (-20°C approximately) to keep the temperature.

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.

The distribution of samples was subcontracted to a courier previously homologated by TestQual. The main criteria being the courier's delivery time to ensure the receipt of the sample is correct in the participant's facilities.

In addition to this, TestQual established other characteristics important for a courier like shipping management (tracking, notifications, exceptions), and ensuring the delivery conditions are proper (low breakage/lost ratio, keeping of cold chain, required documentation), always checking and evaluating they are complying with TestQual's requirements.

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 the samples instructions carefully and follow them, as it can help to correctly conserve the sample and increase the reproducibility of the analysis. You can request a digital copy of this document (sample instructions) by letting us know through any communication channel.

6. CONCENTRATION RANGES, SIGMA OBJECTIVE AND ANALYTES

For this proficiency tests the range of concentration for the target analytes might be between **10** and **300 µg/Kg approximately**. This range is not absolute and depending on the various factors some or no analyte might be within this range. This range is given to provide participants a sense of the concentration ranges we usually work and expect in these kind of proficiency tests (combination of analyte/matrix).

The maximum concentration can vary depending on the MRL of the analytes in the matrix.

The **sigma objective ($\hat{\sigma}$)** which works in this scheme can be checked on section 9 of this protocol.

In this proficiency test for each group of analytes, a percentage of analytes will be present (ranging from >1% (the % is determined internally in our procedure) to possibly all of the analytes of the list). Therefore, this PT should be useful to check each of the groups, possibly in different degrees, depending on the number of selected/present analytes.

In the following table you can check the groups of analytes for this proficiency test, below you can find the lists of possible positives and how the website and the results are expected to be sent:

Group of analytes	Name
1	Multiresidues pesticides,
2	Fosetil, phosphonic acid
3	Chlorate and perchlorate
4	Glyphosate

The list of possible multi- residues pesticides in the matrix are from the list below:

Abamectin
Acetamiprid

Cymoxanil
Cypermethrin

Fipronil
Fludioxonil

Permethrin
Pirimicarb

Acrinathrin	Cyproconazole	Fluquinconazole	profenofos
Aldrin	Cyprodinil	Flusilazole	Propanil
Atrazine	Deltamethrin	Flutriafol	Propargite
Avermectin B1a	Diazinon	HCH-Alpha	Propoxur
Avermectin B1b	Diclofop-methyl	HCH-Beta	Pyraclostrobin
Azinphos-ethyl	Dieldrin	HCH-Gamma (lindane)	Pyridaben
Azoxystrobin	Difenoconazole	Hexythiazox	Pyrimethanil
Benfluralin	Diphenylamine	Imazalil	Pyriproxyfen
Bifenthrin	Dodine	Imidacloprid	Quintozone
Boscalid	Endosulfan-alpha	Indoxacarb	Spiroxamine
Bromophos-ethyl	Endosulfan-beta	Kresoxim-methyl	Tebuconazole
Bromopropylate	Endosulfan-sulfate	Lambda-cyhalothrin	Tebufenozide
Bupirimate	Endrin	Linuron	Tecnazene
Buprofezin	Epoxiconazole	Metazachlor	Tefluthrin
Carbaryl	Etioazole	Metribuzin	Terbuthylazine
Chlorpropham	Famoxadone	Myclobutanil	Tetraconazole
Chlorpyrifos	Fenazaquin	Nitrofen	Thiacloprid
Chlorpyrifos Methyl	Fenbuconazole	Oxyfluorfen	Thiamethoxam
Chlorsulfuron	Fenhexamid	Parathion-ethyl	Triazophos
Clofentezine	fenoxy carb	Parathion-methyl	Ziram
Clothianidin	Fenpropimorph	Pendimethalin	Zoxamide
Cyfluthrin	Fenvalerate		

In addition to the previous, the following analytes will also be available to be quantified and evaluated:

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

- “Fosetyl_AI (Sum)” will be evaluated as “Fosetyl-AI (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)”.
- “Phosphonic Acid” as “Phosphonic acid and their salts”.
- “Fosetyl” as only “Fosetyl” ($C_2H_6O_3P^+$ MW= 109.04)

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”

The results for Chlorate will have to be reported as EU applicable legislation Commission Regulation (EU) 2020/749 of 4 June 2020 amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chlorate in or on certain products

- “Chlorate” as “Chlorate”

The results for Chlorate will have to be reported as EU applicable legislation Commission Regulation (EU) 2020/685 of 20 May 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of perchlorate in certain foods (Text with EEA relevance)

- “Perchlorate” as “Perchlorate”

7. RESULTS EXPRESSION

Each participant laboratory must analyse the sample received according to their routine procedure and fill up the RESULTS form, facilitated by TestQual along the sample instructions with just one value per analyte/parameter.

The results should be expressed in $\mu\text{g/Kg}$. The number of significant figures and the units are to be chosen by laboratories and will be displayed in the report as received by the participant.

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.

You are allowed to modify results within the stipulated period range, until the deadline to submit results, included. For any modification you will be required to open an issue in your client area for this PT and send the required modifications through that communication channel or by contacting via email with the coordinator.

8. 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 valid values with a test using the kernel's density representation, being explained in the final report which is the followed procedure in case there is more than one distribution.

If enough valid results (determined in our internal procedure) are provided and the uncertainty is negligible the **assigned value (X)** will be 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.

If the uncertainty of the assigned value obtained through consensus cannot be neglected, the evaluation will be done with z'-score or informative. If not sufficient results are obtained for the consensus, TestQual, alternatively would evaluate the results against the characterization done to the samples (design according to ISO 13528 into force), this way, the participants would be evaluated against an independent value from their own results, since the lower the number of results are used to obtain the assigned value, the higher is the chance of not identifying necessary action signals for participants that have/had an unknown bias.

Both the assigned value obtained from robust statistic and the characterization value are compared between them, this way we check that there are not sustainable differences. In case of detecting a sustainable difference between them, the consensus value will be used for the evaluation.

Likewise, and as mentioned, the characterization value will be compared to the participants' consensus and only used if there are not sustainable differences between assigned values. If the assigned values are too different, no evaluation or informative evaluation would be issued, explaining the reason in the report.

The **standard uncertainty (u_x)** when the assigned value is obtained from the consensus it is calculated using robust statistics from the following formula:

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. When the assigned value is obtained from the spiking done, the uncertainty is calculated accounting all sources of uncertainty according to our internal procedure and according to ISO 13528 into force.

The following condition must be fulfilled in order to discard the contribution of the uncertainty:

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

The **standard deviation for proficiency assessment**, also named **target standard deviation, ($\hat{\sigma}$)**, comes from this formula:

$$\hat{\sigma} = b_i \cdot X$$

Being $b_i = \%_{tRSD} / 100$, and $\%_{tRSD}$ is the target relative standard deviation.

In this case, the assigned relative standard deviation is **25 %**.

This value has been chosen according to the experience of the organizer in this and similar proficiency test which procedure of the analysis similar to the parameters of this PT.

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

In case the inequation $u_x \leq 0,3 \hat{\sigma}$ is not fulfilled, the participants of the scheme will be informed in the report that the uncertainty is not negligible. For the parameters/analytes in which this situation occurs, the following calculation will be made:

$$z'\text{-score} = (x_i - X) / \sqrt{\hat{\sigma}^2 + U_x^2}$$

Where x_i is the value reported by the laboratories, X is the assigned value, $\hat{\sigma}$ is the target standard deviation for each analyte and U_x is the uncertainty of the assigned value.

The criteria for defining the z' -score values are:

	$ z' $	≤ 2	Satisfactory
$2 <$	$ z' $	≤ 3	Questionable
	$ z' $	> 3	Unsatisfactory

The z' -score is a underestimation of the z-score, for this reason, for those analytes in which the uncertainty of the assigned value cannot be neglected and a z' -score is issued, it will be accompanied by the percentual difference against z-score, this way participants should be able to complete evaluate their performance.

The evaluation could be informative if the difference between scores surpasses the limit contemplated in our procedure. If any analyte or evaluation is informative it will be indicated in the report through marking and a legend.

False negatives: Any analyte not reported in the results that is in the sample above the limit of quantification previously established for this proficiency test by the organization and above the LOQ of the participant laboratory (**10 $\mu\text{g/Kg}$**). TestQual assigns to all false negatives a result equal to half the laboratory limit of quantitation (LOQ/2). If no LOQ is available, it will be assumed to be "0" for the calculation.

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 $\mu\text{g/Kg}$**).

Testing for sufficient homogeneity:

Ten samples will be chosen at random and sent to be analysed by TestQual's subcontracted laboratory in duplicate in repeatability conditions.

Once received the results, a statistical evaluation will be performed, with the homogeneity evaluation of the Harmonic Protocol published by IUPAC and based in ISO 13528 into force.

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:

$$S_{sam}^2 < c$$

Being S_{sam}^2 the estimated sampling standard deviation, obtained from the variance of the results sums and the experimental estimate of analytical standard deviation (S_{an}), which in turn is obtained from the differences between replicates of the same sample. Lastly, c is the limit value, which is obtained as ISO 13528 states, it takes into account two constants, obtained from two significance groups, which are multiplied with a term related to the target standard deviation and the S_{an} .

If $S_{sam}^2 < c$ is true, then the lot prepared will be considered sufficiently homogeneous and only then it would be distributed. With this test what we achieve is to check if the intra-sample deviation is lower than the inter-sample deviation.

If the results from the homogeneity test does not meet the criteria TestQual would communicate any change required in the proficiency test (new lot will be prepared, new calendar, etc.).

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 to 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 proficiency test 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.

9. EVALUATION REPORT/S

Once received and statistically evaluated all the laboratories' results, TestQual will send a global report that summarizes the participation of each laboratory.

This global report will be received by the laboratories via e-mail in PDF format or an email notifying that the report is now available to 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.

Likewise, the participant can request an “individual” report for each of the groups of the analytes of their interest, the “individual” report is only a summary, which will reference the global report to complete the required information.

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, there will be a 60-day period to communicate any appellation.

10. 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

11. BIBLIOGRAPHY AND REFERENCES OF INTEREST

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.

ISO13528:2022. Statistical methods for use in proficiency testing by interlaboratory comparison.

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

EU Pesticides database (v.2.2) Search Pesticides residues

SANTE/11312/2021 (Implemented by 01/01/2022) Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

COMMISSION REGULATION (EU) 2020/749 of 4 June 2020 amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for **CHLORATE** in or on certain products
OJ L 178, 8.6.2020, p. 7–20

COMMISSION REGULATION (EU) 2020/685 of 20 May 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of **PERCHLORATE** in certain foods
OJ L 160, 25.5.2020, p. 3–5

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 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

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