RAPPORTI ISTISAN 26|9

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

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AMBIENTE E SALUTE

ISTITUTO SUPERIORE DI SANITÀ

Results of the Proficiency Test 2025 on the determination of aflatoxins in pistachios

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2026, 41 p. Rapporti ISTISAN 26/9

Every year, the Italian National Reference Laboratory for Mycotoxin and Plant Toxins at the Istituto Superiore di Sanità (the National Institute of Health in Italy) organizes proficiency test trials for the Italian network of the Official Laboratories. In 2025, a study for the determination of aflatoxins in pistachios was organized in cooperation with the Direzione Operativa Chimica dell'Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri". Laboratories invited to participate in this study are Italian Official Control Laboratories and European laboratories. The majority of participants obtained a satisfactory performance (z-score, ζ-score).
Keywords: Aflatoxins; Pistachios; Proficiency test

Istituto Superiore di Sanità
Risultati del circuito interlaboratorio 2025 sulla determinazione delle aflatossine nei pistacchi.
Francesca Debegnach, Dario Lucchetti, Emanuela Gregori, Simona Mauro, Martina Enza Grieco, Katia Russo, Barbara De Santis
2026, 41 p. Rapporti ISTISAN 26/9 (in inglese)

Il Laboratorio Nazionale di Riferimento per le Micotossine e per le Tossine Vegetali Naturali presso l'Istituto Superiore di Sanità organizza almeno una prova valutativa ogni anno destinata al circuito di laboratori ufficiali del Servizio Sanitario Nazionale. Nel 2025, in collaborazione con la Direzione Operativa Chimica dell'Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", è stata organizzata una prova valutativa per la determinazione delle aflatossine nei pistacchi. I laboratori invitati sono stati i laboratori italiani del controllo ufficiale e alcuni laboratori europei. La maggioranza dei partecipanti ha ottenuto prestazioni soddisfacenti in termini di *z-score* e ζ-*score*.
Parole chiave: Aflatossina; Pistacchi; Prova valutativa

Per informazioni su questo documento scrivere a: lnr-micotossine-tvn@iss.it

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Citare questo documento come segue:
Debegnach F, Lucchetti D, Gregori E, Mauro S, Grieco ME, Russo K, De Santis B. *Results of the Proficiency Test 2025 on the determination of aflatoxins in pistachios.* Roma: Istituto Superiore di Sanità; 2026. (Rapporti ISTISAN 26/9).

Legale rappresentante dell'Istituto Superiore di Sanità: *Rocco Bellantone*
Registro della Stampa - Tribunale di Roma n. 114 (cartaceo) e n. 115 (online) del 16 maggio 2014

Direttore responsabile della serie: *Antonio Mistretta*
Redazione*: Sandra Salinetti, Annalisa D'Angelo*
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INTRODUCTION

In accordance with Article 37 of Regulation (EU) 2017/625, all laboratories involved in official control analyses must provide evidence of their analytical skills, for a specific test or for groups of tests. Furthermore, it is established that the official laboratories designated by the competent authority must be evaluated and accredited according to the international standard ISO/IEC 17025:2017. Among the various requirements set by this ISO standard, participation in Proficiency Testing (PT) is considered an essential tool for verifying the performance of a laboratory's processes. It must be ensured for at least the tests for which accreditation has been requested or granted. As part of the quality assurance activities, laboratories are required to provide evidence of both internal and external quality control measures. Internal quality control may include the use of control charts, reference materials, evaluation of the recovery factor, and repeatability testing, all of which should be systematically implemented. External quality control, on the other hand, can be achieved through the participation in interlaboratory trials and/or the exchange of samples with other laboratories.

The use of validated methods, together with regular participation in the PTs schemes, represent a fundamental components of a laboratory quality assurance system. Participation in PT exercises enables an independent assessment of the laboratory's competence and its compliance with the requirements reported in Regulation (EU) 2017/625.

This PT has been designed according to the IUPAC (International Union of Pure and Applied Chemistry) Technical Report "International harmonized protocol for the proficiency testing of analytical chemistry laboratories" and to ISO 13528:2022, and in compliance with the standard ISO/IEC 17043:2023. The PT 2025 is on the determination of aflatoxins in pistachios and it has been coded as PT 2025 AFs in pistachios.

Mycotoxins are natural contaminants of food and feed, mainly produced by molds belonging to the genus *Aspergillus*, *Penicillium* and *Fusarium*. The number of mycotoxins known to exert toxic effects on human and animal health is constantly increasing, as are the legislative provisions adopted to control their presence in food and feed. Among mycotoxins, aflatoxins, and aflatoxin B1 in particular, are the most toxic hazards and the risk related to their presence in several food products such as cereals, nuts and dried fruits, is considered the highest risk of food origin and a serious threat for human health.

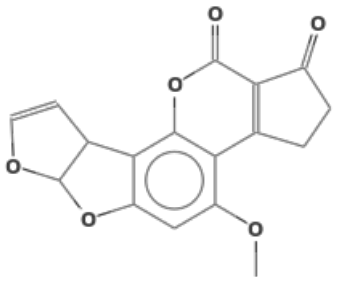
Aflatoxins are primarily produced in the field under conditions of high temperature and humidity; however, inadequate post-harvest handling and poor storage conditions can further promote fungal growth and toxin production, representing a critical phase for the final contamination of food and feed.

Human exposure to AFB1 and to the sum of aflatoxins (AFB1, AFB2, AFG1 and AFG2) has been clearly demonstrated by the detection of these compounds, or their metabolites, in biological samples such as blood, urine. The main dietary sources include maize and other cereals, tree nuts, oilseeds such as peanuts, spices and dried fruits. Figure 1 shows chemical information about each of the aflatoxins.

AFB1 is widely recognized as one of the most toxic and carcinogenic mycotoxins. Its primary target is the liver, where it can cause both acute and chronic toxicity, and significantly increases the risk of hepatocellular carcinoma. This risk is even higher in individuals with chronic hepatitis B infection. On the basis of strong evidence from both human and animal studies, the International Agency for Research on Cancer has classified AFB1 as carcinogenic to humans (Group 1).

According to most recent European Food Safety Authority (EFSA) scientific opinion, due to the genotoxic and carcinogenic nature of AFB1, the risk assessment is carried out using the

Margin of Exposure (MOE) approach. For this purpose, a benchmark dose lower confidence limit associated with a 10% increase in liver cancer incidence (BMDL10) of 0.4 μg/kg body weight per day has been identified, based on animal studies. Risk characterization with estimated dietary exposure indicates a potential concern for human health, particularly for vulnerable population groups especially in areas where aflatoxin contamination is not adequately controlled.



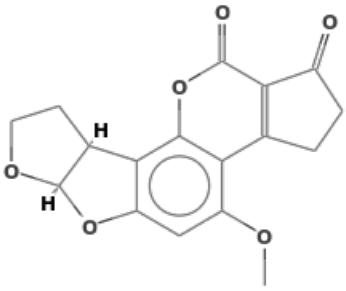
Aflatoxin B1

Formula: $C_{17}H_{12}O_6$

Molecular weight: 312.2736

CAS Registry Number: 1162-65-8

IUPAC Name: (3S,7R)-11-methoxy-6,8,19-trioxapentacyclo[10.7.0.02,9.03,7.013,17]nonadeca-1,4,9,11,13(17)-pentaene-16,18-dione



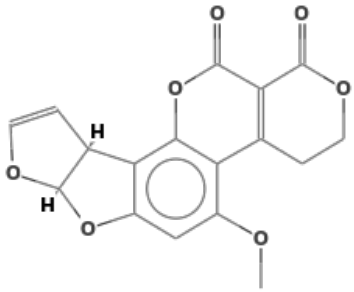
Aflatoxin B2

Formula: $C_{17}H_{14}O_6$

Molecular weight: 314.289

CAS Registry Number: 7220-81-7

IUPAC Name: (3S,7R)-11-methoxy-6,8,19-trioxapentacyclo[10.7.0.02,9.03,7.013,17]nonadeca-1,9,11,13(17)-tetraene-16,18-dione



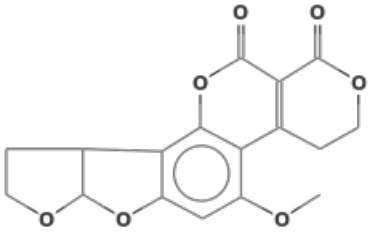
Aflatoxin G1

Formula: $C_{17}H_{12}O_7$

Molecular weight: 328.2730

CAS Registry Number: 1165-39-5

IUPAC Name: (3S,7R)-11-methoxy-6,8,16,20-tetraoxapentacyclo[10.8.0.02,9.03,7.013,18]icosa-1,4,9,11,13(18)-pentaene-17,19-dione



Aflatoxin G2

Formula: $C_{17}H_{14}O_7$

Molecular weight: 330.2889

CAS Registry Number: 7241-98-7

IUPAC Name: (3S,7R)-11-methoxy-6,8,16,20-tetraoxapentacyclo[10.8.0.02,9.03,7.013,18]icosa-1,9,11,13(18)-tetraene-17,19-dione

Figure 1. Chemical information about aflatoxins

ORGANIZATION OF THE PROFICIENCY TESTING

Preparation of the test material

The PT test material consisted of a finely ground pistachio naturally contaminated with aflatoxin B1, B2 and G1. The material was homogenized through mixing and grinding using a 0.50 mm sieve and subsequently aliquoted into 50 mL conical centrifuge tubes. The material was then tested for homogeneity and stability.

Homogeneity of the test material

The homogeneity of the material was evaluated in November 2024. To verify homogeneity, 8 conical tubes containing the PT material were randomly selected and analyzed in duplicate. The evaluation of test material homogeneity was performed in accordance with the international harmonized protocol for proficiency testing of analytical laboratories.

Shipment of the test material and request to the participants

The PT was announced at national level by email on 26th March 2025, and the invitation was subsequently extended to European participants via email on 18th April 2025. Participants were free to apply their routine analytical methods. Each laboratory was assigned a unique code to ensure confidentiality and anonymity. The test material was dispatched to participants on 27th May 2025 under controlled conditions (shipped at -18°C). Upon receipt, participants in the accompany instruction were instructed to store the sample at -18°C until the time of analysis.

Each participant received:
- a pistachios sample in a 50 mL conical test tube;
- a PT cover letter;
- an acknowledgement of receipt form;
- a questionnaire on the analytical method used to carry out the PT;
- a results form for sending the results;
- the code assigned to the laboratory.

Copy of the documents sent to the participants is included in Appendix A.

Participants were asked to:
a) send back the results no later than 30/09/2025;
b) provide the results in µg/kg;
c) correct the results for the recovery factor;
d) indicate the value of the recovery factor (expressed in %);
e) associate the measurement uncertainty (expressed in µg/kg) to the analytical result and indicate the coverage factor (k) and the confidence level expressed in %;
f) provide the value of the limit of quantification (LOQ) of the applied method (expressed in µg/kg).

Statistical evaluation of the results

The statistical evaluation of the results provided by the participants was carried out in accordance with the IUPAC Technical Report ("The international harmonized protocol for the proficiency testing"), the ISO 13528:2022 and the Analytical Methods Committee publications on robust statistics.

Assigned value, robust standard deviation and associated uncertainty

The assigned value was established as the consensus value derived from participants' results. In PT schemes, the consensus value is estimated as the robust mean, $\widehat{X}$. Both the $\widehat{X}$ and the robust standard deviation, $\widehat{\sigma}_{rob}$, were calculated using algorithm A (ISO 13528:2022).

The uncertainty of the assigned value (u) was estimated from $\widehat{\sigma}_{rob}$ according to the following formula:

$$u = 1.25 \times (\frac{\hat{\sigma}_{rob}}{\sqrt{n}})$$

where:

- u is the uncertainty of the consensus value, μg/kg;
- n is the number of values used to calculate the consensus value;
- $\hat{\sigma}_{rob}$ is the estimate of the standard deviation of the consensus value resulting from robust statistics, μg/kg.

The suitability of the standard deviation for proficiency assessment (σ_{PT}) was verified to ensure that the uncertainty of $\hat{X}$ does not adversely affect the evaluation of participant performance, including potential effects related to analyte instability in the PT material. Specifically, the following criterion was applied:

$$u \leq 0.3\ \sigma_{PT}$$

If this condition is satisfied, the assigned value ($\widehat{X}$) and $\widehat{\sigma}_{rob}$ can be used directly for performance evaluation.

If this condition is not met, and the following conditions applies:

$$0.3\ \sigma_{PT} < u \leq 0.7\ \sigma_{PT}$$

a modified standard deviation for proficiency assessment (σ'_{PT}) is used, calculated as follows:

$$\sigma'_{PT} = \sqrt{\sigma_{PT}^2 + u^2}$$

Standard deviation for proficiency assessment and unimodality of data

For the evaluation of laboratory performance, a fixed standard deviation for proficiency assessment (σ_{PT}) equal to 25% of the concentration value was applied. The rationale behind this σ_{PT} choice is provided by the European Union Reference Laboratory for Mycotoxins and Plant Toxins (EURL-MP) which recommends this value as fit for purpose value, reflecting current analytical capabilities and best practices for the determination of mycotoxins and plant toxins in food and feed. The σ_{PT} defines the acceptable dispersion of results within the interlaboratory comparison and is applied regardless of the analyte, matrix, or concentration level.

To assess the distribution of the reported results, a kernel density plot was examined using a normal density and a bandwidth set at $0.75\sigma_{PT}$, in order to verify the presence of a unimodal and approximately symmetric distributions in the dataset of the reported results. When unimodality is confirmed, or when a secondary mode contributes less than approximately 5% to the overall kernel density distribution, it is considered appropriate to estimate the robust mean, $\widehat{X}$, and the corresponding standard deviation ($\widehat{\sigma}_{rob}$).

z-score, *proxy*-z-score and ζ-score calculation

The performance of individual laboratories is evaluated in terms of z-score. Where available the uncertainty value, also ζ-score was calculated in accordance with ISO 13528:2022 and the IUPAC international harmonized protocol for proficiency testing of analytical laboratories.

Participants' z-score values are calculated by applying the following formula:

$$\text{z-score} = \frac{(x_{lab} - \widehat{X})}{\sigma_{PT}}$$

where:

- x_{lab} is the result provided by the laboratory, μg/kg;
- $\widehat{X}$ is the assigned value, μg/kg;
- σ_{PT} is the target standard deviation for the PT, μg/kg.

The alarms signal and the actions signal are established for the intervals $X \pm 2\sigma_{PT}$ and $X \pm 3\sigma_{PT}$, respectively. In fact, considering a normal distribution of the results, 95% of the observations fall within the interval $\mu \pm 2\sigma_{PT}$ and 99.7% of the observations fall within the interval $\mu \pm 3\sigma_{PT}$ Thus, there is a 1 in 20 chance that a laboratory result is acceptable but not in the $\mu \pm 2\sigma_{PT}$ range, and only a 1 in 300 chance that the laboratory bias is greater than $3\sigma_{PT}$ by chance.

In conclusion:

- if the participant's z-score is $|z| \leq 2$ the result is considered satisfactory (the z-score value is within the alarm signal range);
- if the z-score score is $2 < |z| < 3$, the result is questionable (the z-score value is outside the alarm signal range, but is still within the action signal range);
- if the z-score score is $|z| \geq 3$, the result is considered unsatisfactory (the z-score value is outside the action signal range).

The performance using the *proxy* z-score is calculated when a result is reported as below the LOQ and expressed as <LOQ or "< x µg/kg" with the LOQ value explicitly stated. The proxy z-score are calculated by applying the following formula:

$$\textit{proxy}\text{-z-score} = \frac{(x_{lab} - \hat{X})}{\sigma_{PT}}$$

where:

- x_{lab} is the laboratory result reported as <x µg/kg;
- $\hat{X}$ is the assigned value (µg/kg);
- σ_{PT} is the standard deviation for proficiency testing (µg/kg).

Proxy z-scores are not included in the graphical presentation of participants' z-scores.

The evaluation interpretations are as follows:

- if the participant's proxy z-score ≤−3 on the basis of the reported LOQ, the laboratory should be capable of detecting and quantifying the analyte. The result is therefore classified as a false negative (FN) and interpreted as unsatisfactory performance;
- if the participant's −3<*proxy* z-score<−2 given the reported LOQ, the laboratory is likely capable of detecting and quantifying the analyte. The result is classified as a false negative (FN) and interpreted as questionable performance;
- if the participant's −2≤ *proxy* z-score≤2:
  - (A) (−2 to 0) considering both the assigned value and the reported LOQ, the result cannot be classified as a false negative;
  - (B) (0 to 2) the LOQ lies within the range considered analytically feasible, based on the performance achieved by other laboratories;
- if the participant's 2<*proxy* z-score<3, the LOQ is high relative to analytically feasible performance. The laboratory is advised to evaluate the possibility of reducing the LOQ;
- if the participant's *proxy* z-score≥3, the LOQ is excessively high compared to analytically feasible performance. The LOQ associated with the analytical method cannot be regarded as acceptable.

Participants' ζ-score values are calculated by applying the following formula:

$$\zeta\text{-score} = \frac{(x_{lab} - \hat{X})}{\sqrt{u_{lab}^2 + u^2}}$$

where:

- x_{lab} is the result provided by the laboratory, µg/kg;
- $\hat{X}$ is the assigned value, µg/kg;
- u_{lab} is the standard uncertainty provided by the laboratory, µg/kg;
- u is the standard uncertainty associated with the assigned value, µg/kg.

The warning and action intervals and the evaluation (satisfactory, questionable and unsatisfactory) are completely similar to those seen for the z-score.

The ζ-score was used as an indication of the consistency of the uncertainty provided by the participant (u_{lab}) with the observed deviation from the assigned value (u). Unsatisfactory ζ-score values may indicate an underestimation of the uncertainty or a large deviation of the result from the assigned value, indicating an uncertainty value that is inconsistent with the uncertainty of the assigned value. Unsatisfactory values of z-score and ζ-score may occur when the uncertainty

values are not in agreement with the observed deviation from the assigned value, so it is useful to consider together the values of z and ζ-score.

Follow-up

In cases where the participating laboratory obtains a result considered questionable or unsatisfactory, resulting from a score of $2<|z|<3$ or z-score $|z|\geq 3$, the National Reference Laboratory (NRL) will contact the laboratory of the Italian network to share the root cause analysis which may have led to the unsatisfactory result and the corresponding planned corrective actions.

The NRL has planned a follow-up questionnaire which requests the planned and implemented actions and the timescales required to verify the adequacy of the corrective action planned or undertaken. If necessary, a new aliquot of the PT test material may be sent upon request.

RESULTS AND DISCUSSION

Homogeneity of the test material

The material was found to be sufficiently homogeneous for PT. The results of the homogeneity study are presented in Table 1, where:

- x_{hom} homogeneity value;
- S_{an} Analytical standard deviation;
- S^2_{sam} Sampling variance;
- σ_{all} limit standard deviation for sufficient homogeneity;
- $c = F1\sigma^2_{all} + F2S^2_{an}$ (F1 and F2, factors used for homogeneity test for m samples analyzed in duplicate).

Table 1. PT 2025 AFs in pistachios: results of the homogeneity test

Parameters	AFB1	AFB2	AFG1	AFs
Number of independent replicates (duplicates)	8	8	8	8
x_{hom} (µg/kg)	2.00	0.22	0.50	2.72
Cochran test	0.33	0.37	0.23	0.33
S_{an} (µg/kg)	0.07	0.01	0.04	0.09
S^2_{sam}	0.0025	0.0001	0.0007	0.0038
σ_{all}	0.13	0.01	0.03	0.18
Critical value, c	0.04	0.001	0.004	0.08
S^2_{sam}<critical value?	YES	YES	YES	YES

Assigned value and associated uncertainty

The consensus value in this PT was obtained by calculating the robust mean, $\hat{X}$. The uncertainty of the assigned value (u) was calculated from $\hat{\sigma}_{rob}$. The uncertainty value compared with the value of σ_{PT} satisfied the criterion $u \leq 0.3\ \sigma_{PT}$ for AFB1 and total aflatoxins (AFs). For AFB2 and AFG1, having satisfied the criterion $0.3\ \sigma_{PT} < u \leq 0.7\ \sigma_{PT}$, the σ'_{PT} was calculated.

The parameters obtained for the characterization of the method, assigned value with the robust standard deviation and standard uncertainty, are shown in Table 2.

Table 2. PT 2025 AFs in pistachios: assigned value with relative standard deviation,standard uncertainty and PT target standard deviation obtained with the data of the PT participants

Parameters	AFB1	AFB2	AFG1	AFs
Assigned value, $\hat{X}$ (µg/kg) (algorithm A)	1.50	0.18	0.26	1.84
Assigned value robust standard deviation, $\hat{\sigma}_{rob}$ (µg/kg)	0.38	0.06	0.07	0.36
Assigned value standard uncertainty, u (µg/kg)	0.10	0.02	0.03	0.10
PT target standard deviation, σ_{PT} (µg/kg)	0.37	0.05*	0.07*	0.46

*σ'_{PT}

Identification of the outliers

Before proceeding with the calculation of the z-score and ζ-score values, a verification of the hypothesis of normal distribution of the data was made to check for the presence of outliers. In order to confirm the normal trend and to exclude any deviation, the visual verification of the kernel density plot, the box plot and the Q-Q plot was carried out.

The graphs of the kernel density (with bandwidth equal to $0.75\sigma_{PT}$), gave evidence of unimodality for AFB1 and AFs, while the mention of the second mode contributing with less than 5% to the density distribution, was observed for AFB2 and AFG1.

This has encouraged the use of robust statistics for data processing (calculation of the robust mean and its standard deviation).

The calculation of the assigned value (the robust average) and the robust standard deviation were performed on the entire dataset of the results delivered by the laboratories.

Figures 2-5 show the kernel density plot, box plot and Q-Q plot obtained for AFB1, AFB2, AFG1 and AFs, respectively.

Results of the participants

The PT has joined 25 laboratories of which:

– 9 Italian laboratories of the official control network;
– 16 European laboratories recruited through the EURL-MP.

The list of laboratories that have joined the PT is reported in Appendix B.

All the participant laboratories have sent the duly completed "Acknowledgement of receipt" form confirming the correct receipt of the material for the execution of the PT. Twenty three out of 25 laboratories provided results. The results obtained were sent by the deadline indicated in the PT cover letter.

In addition, the participants completed the questionnaire on the analytical method in which information on sample preparation and aflatoxins determination was requested. Unfortunately, laboratories 2, 7, 20 and 25 did not provide the completed questionnaire.

The information contained in the questionnaires received is reported in Appendix C.

Among the participants who returned the questionnaire, 18 used an accredited method. Regarding the method applied, 2 laboratories used an Enzyme-Linked Immunosorbent Assay (ELISA) method, 7 a High Performance Liquid Chromatography (HPLC) separation coupled with SpectroFluorimetric Detection (HPLC-FLD) while the majority of the participants (11 laboratories) used a Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) system for the determination of the analytes.

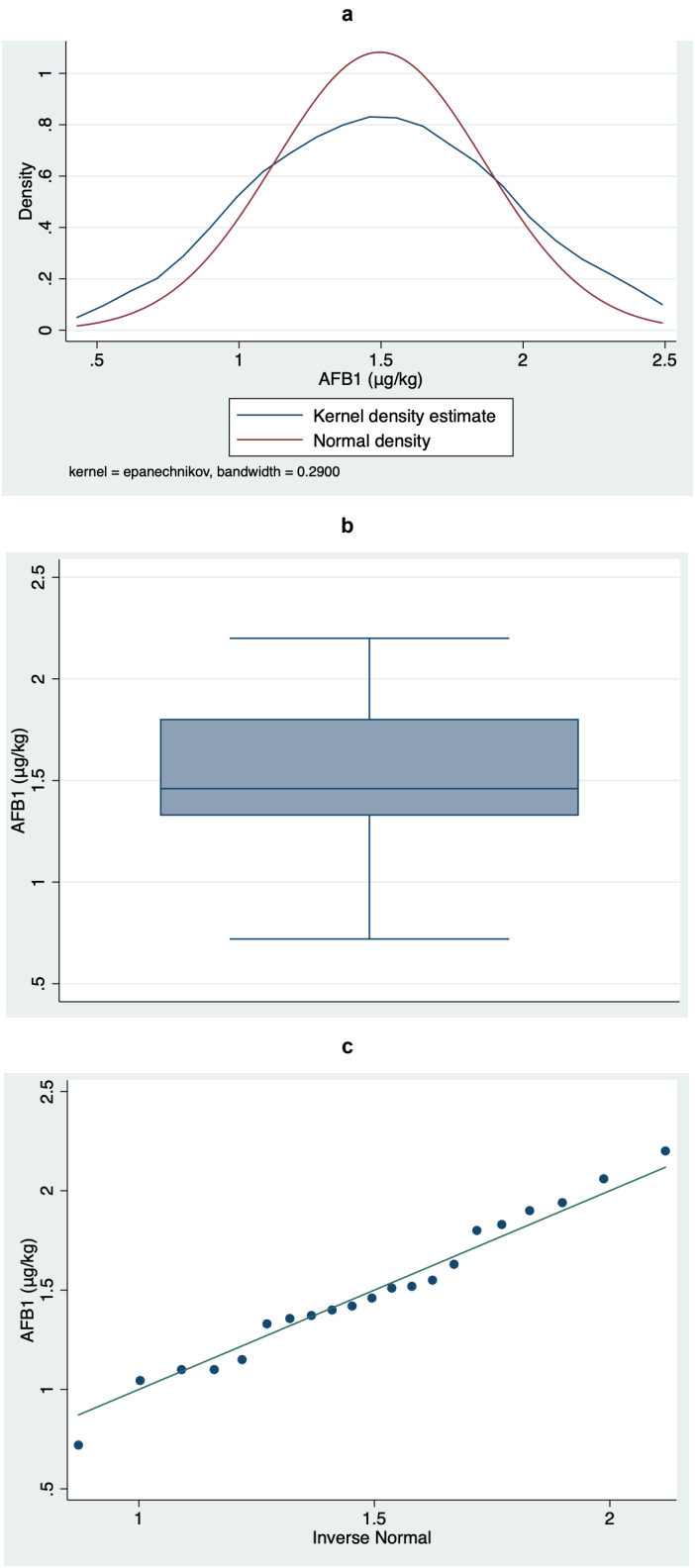


Figure 2. AFB1: a) Kernel density plot, b) box plot, c) Quantile-Quantile plot. PT 2025 AFs in pistachios

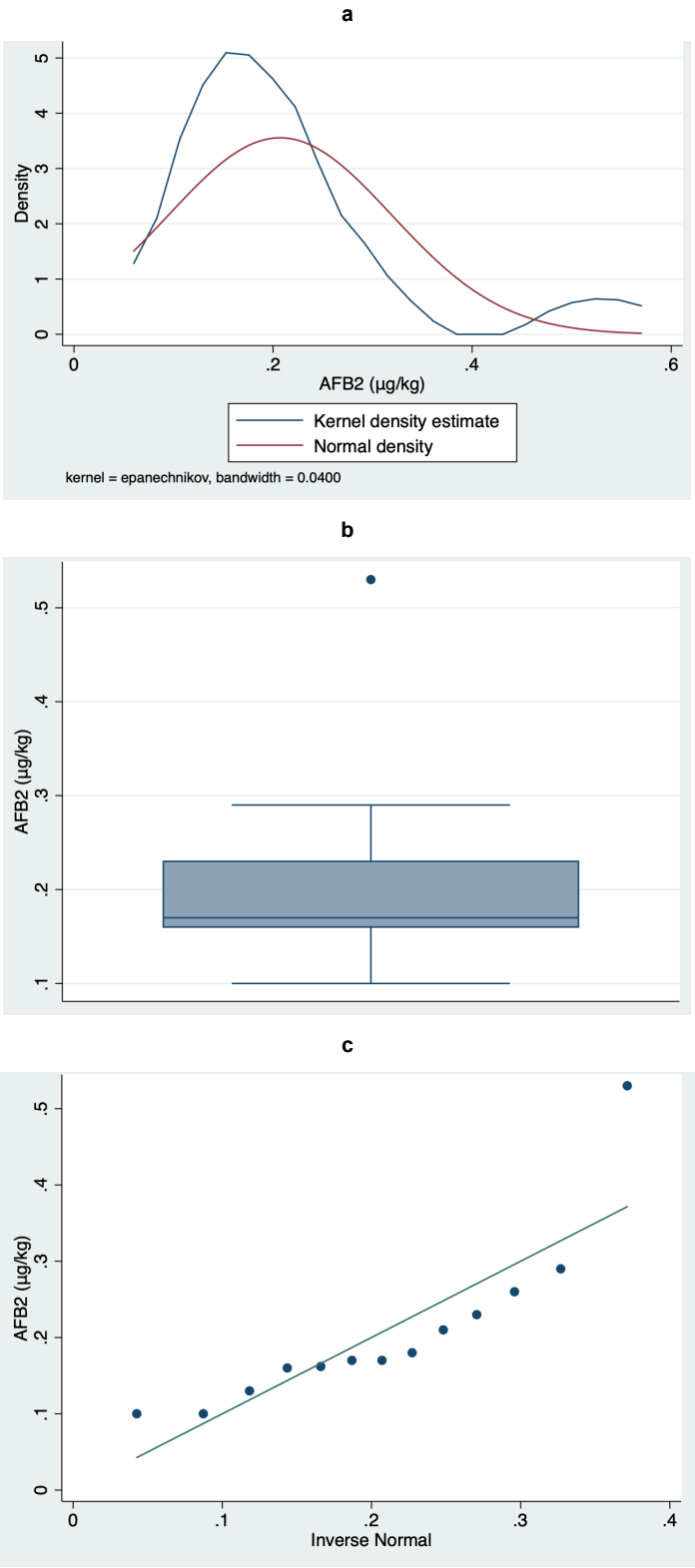
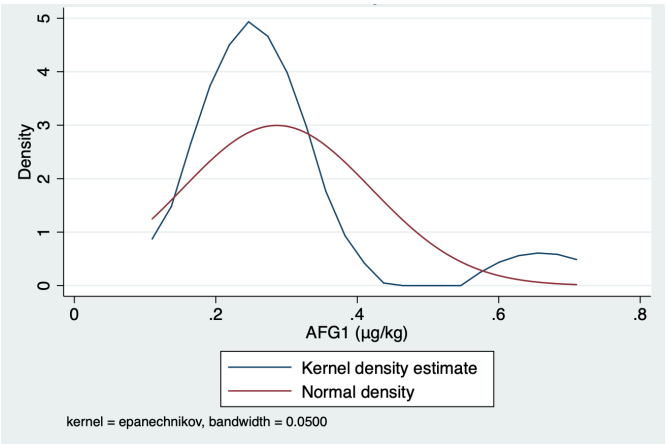


Figure 3. AFB2: a) Kernel density plot, b) box plot, c) Quantile-Quantile plot. PT 2025 AFs in pistachios

a



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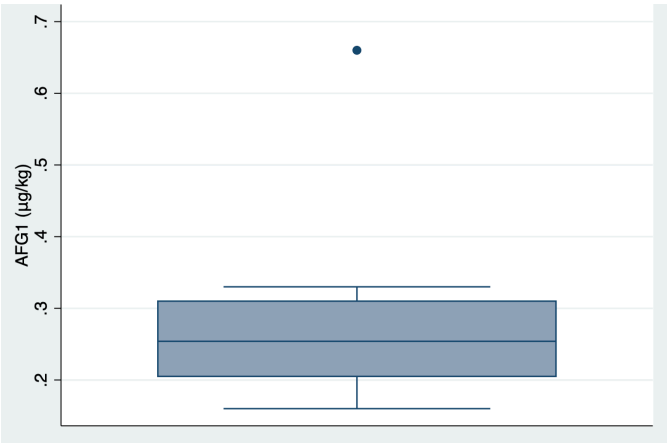

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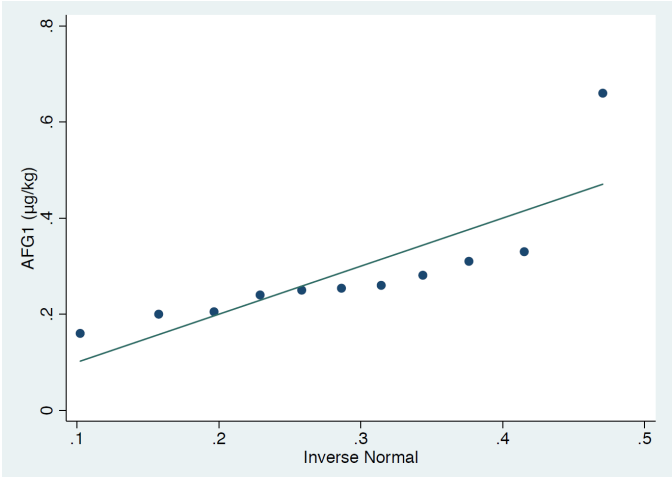

Figure 4. AFG1: a) Kernel density plot, b) box plot, c) Quantile-Quantile plot. PT 2025 AFs in pistachios

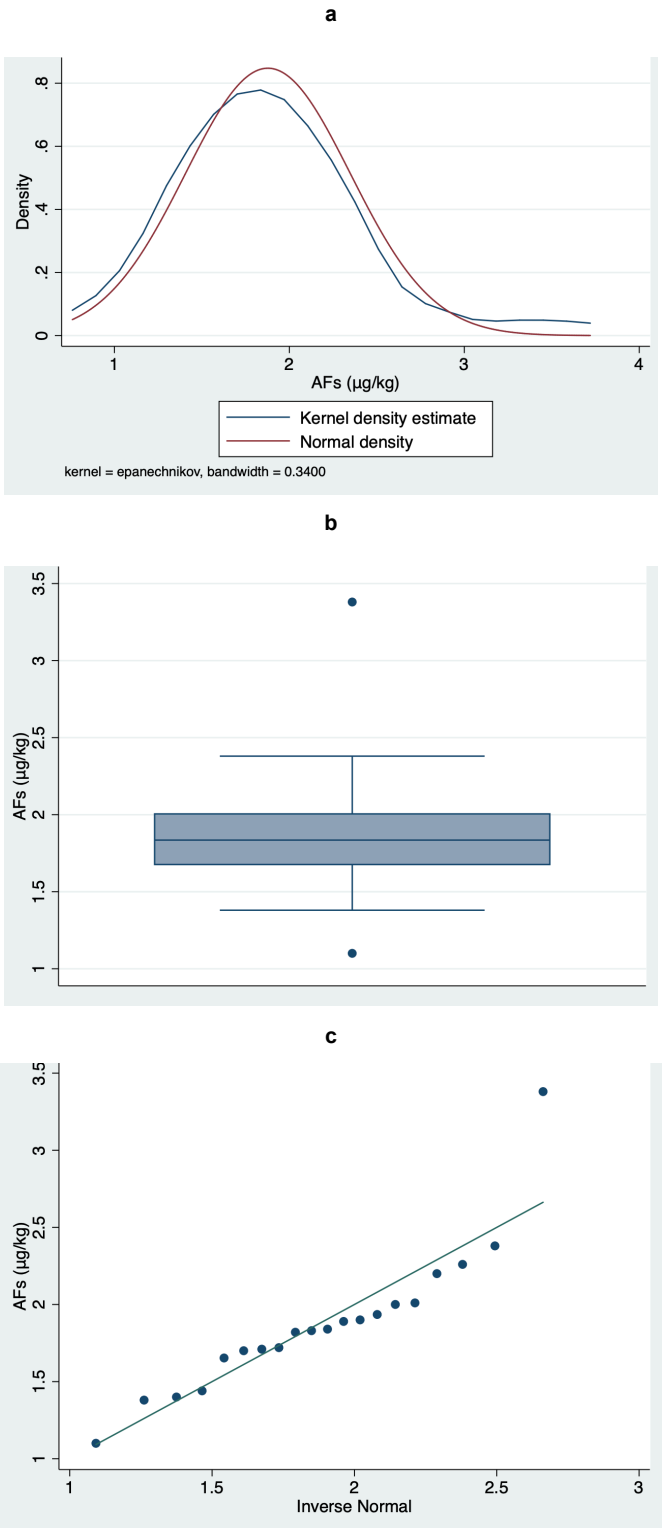

Figure 5. AFs: a) Kernel density plot, b) box plot, c) Quantile-Quantile plot. PT 2025 AFs in pistachios

Aflatoxin B1

The analytical results and the participants' z-score and ζ-score values obtained for aflatoxin B1 are summarized in Table 3.

Table 3. AFB1: analytical results and participants *z-score* and *ζ-score* values. PT 2025 AFs in pistachios

Cod. LAB	AFB1 (µg/kg)	R (%)	Ue (µg/kg)	LOQ (µg/kg)	z-score	ζ-score
2	1.63	70	0.45	1.20	0.4	0.5
3	1.94	110	0.85	0.2	1.2	1.0
4	2.2	72	0.8	0.5	1.9	1.7
5	1.372	103	0.686	0.25	-0.3	-0.4
6	1.33	107	-	0.10	-0.4	-
7	1.4	76.4	0.30	0.25	-0.3	-0.5
8	1.83	77	0.80	0.6	0.9	0.8
9	1.55	120	0.3	0.4	0.1	0.3
10	1.1	100	0.4	0.8	-1.1	-1.8
12	1.357	105.9	0.5748	1.25	-0.4	-0.5
13	1.045	-	0.177	0.1	-1.2	**-3.3**
14	1.510	88	0.50	0.3	0.0	0.0
15	2.06	60	0.27	0.5	1.5	**3.3**
17	1.8	90.5	0.73	0.2	0.8	0.8
18	1.42	76	0.57	0.15	-0.2	-0.3
19	1.519	68	0.456	0.01	0.1	0.1
20	1.46	92	0.263	0.14	-0.1	-0.2
22	1.1	60	0.44	0.1	-1.1	-1.6
23	0.72	105	0.23	0.20	**-2.1**	**-5.0**
24	1.15	94	0.30	0.1	-0.9	-1.9
25	<LOQ	95	0.03	1.6	(0.3)	-
26	1.90	98	0.57	0.6	1.1	1.3
27	<2	-	-	2	(1.3)	-

In addition to the analytical result corrected for recovery, the participants indicated the value of the recovery factor, with the exception of laboratories 13 and 27. Laboratories 25 and 27 reported the result for AFB1 as <LOQ and <2, respectively. These results were not included in the assigned value calculation; however, a *proxy* z-score was calculated using the reported LOQ value as the result. *Proxy* z-scores are for informational purposes only, in Table 3 are indicated as values between brackets. They are not included in the evaluation of results and do not count as satisfactory results. Consistent with the results and information provided, the z-score was calculated for 21 laboratories and the ζ-score for 20, in fact laboratory 6 did not provide the uncertainty value.

Graphically the results, in terms of z-score and ζ-score, are represented in Figures 6 and 7.

Regarding z-score, 95% of the laboratories gave a score with satisfactory values ($|z| \leq 2$) for AFB1, in fact only laboratory 23 had a questionable score ($2 < |z| < 3$). Analyzing the information provided by the laboratory, no possible causes for the questionable performance of the method have been identified.

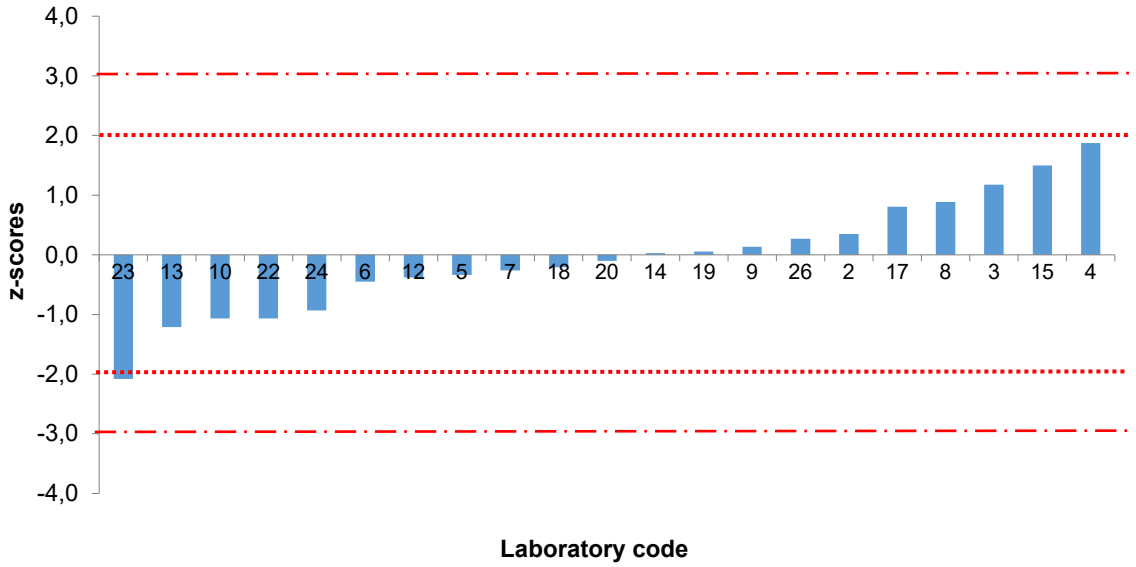

Figure 6. AFB1: z-score values (assigned value x ± U(x) = 1.50 ± 0.21 µg/kg; σ_{PT} = 0.38 µg/kg). PT 2025 AFs in pistachios

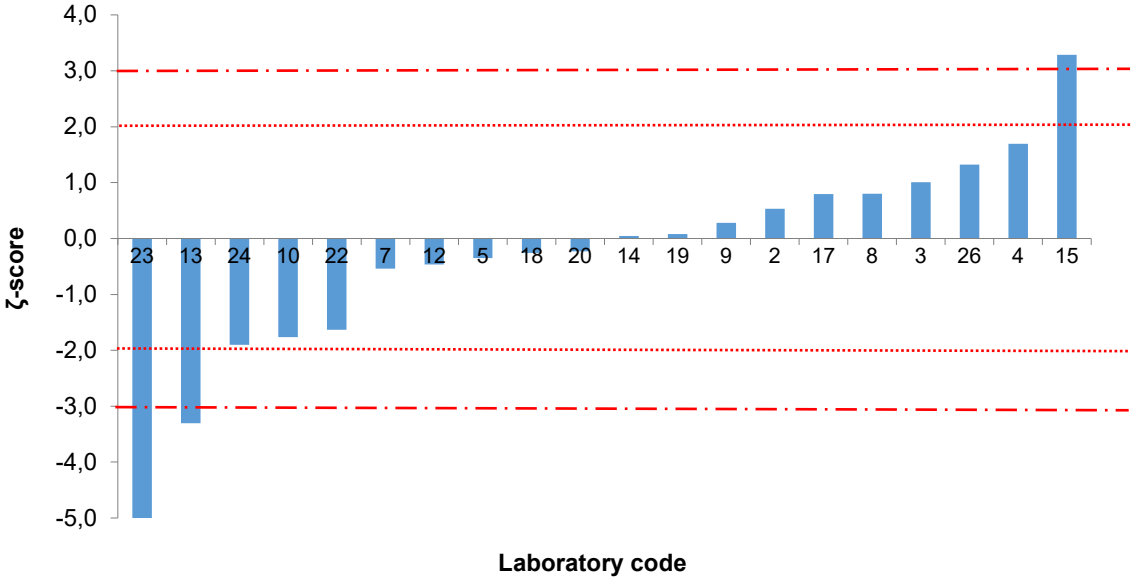

Figure 7. AFB1: ζ-score values (assigned value x ± U(x) = 1.50 ± 0.21 µg/kg; σ_{PT} = 0.37 µg/kg). PT 2025 AFs in pistachios

None of the participants had unsatisfactory scores ($|z| \geq 3$). The two laboratories for which a *proxy* z-score was calculated also had acceptable results; in fact, both participants indicated a LOQ value close to the assigned value. However, in both cases the LOQ value is above the

assigned value, therefore the determination of this level of concentration is out of the concentration range of the applied method for laboratories 25 and 27.

The ζ-score was calculated for 20 laboratories, in fact laboratory 6 did not provide the uncertainty value. Eighty-five% of the laboratories gave a score with satisfactory values ($|\zeta| \leq 2$). None of the participants had questionable results ($2 < |\zeta| < 3$), but three laboratories (13, 15 and 23) had unsatisfactory values ($|\zeta| \geq 3$). Laboratory 23 had a questionable z-score and an unsatisfactory ζ-score, in this case in fact the underestimation of the AFB1 concentration is too strong to be compensated by the uncertainty that accompanies the result. Also, for this method performance no possible causes have been identified analyzing the information provided by the laboratory.

For all other cases, a questionable or unsatisfactory ζ-score value means that the interval defined by the laboratory value and its extended uncertainty ($x_{lab} \pm U_x$) does not include the assigned value.

Aflatoxin B2

The analytical results and the participants' z-score and ζ-score values obtained for aflatoxin B2 are summarized in Table 4.

Table 4. AFB2: analytical results and participants *z-score* and ζ-*score* values. PT 2025 AFs in pistachios

Cod. LAB	AFB2 (µg/kg)	R (%)	Ue (µg/kg)	LOQ (µg/kg)	z'-score	ζ-score
2	0.26	75	0.09	0.42	1.5	1.5
3	0.18	110	0.08	0.2	-0.1	-0.1
4	<LOQ	-	-	0.5	(**6.2**)	-
5	<LOQ	91	-	0.25	(1.3)	-
6	-	-	-	-	-	-
7	0.29	82.2	0.08	0.25	**2.1**	**2.3**
8	<LOQ	80	-	0.2	(0.3)	-
9	0.17	115	0.0	0.1	-0.3	-
10	<LOQ	100	-	0.8	(**12.1**)	-
12	<1.25	-	-	1.25	(**20.9**)	-
13	0.1	-	0.016	0.05	-1.6	**-3.6**
14	0.170	87	0.06	0.3	-0.3	-0.4
15	0.53	60	0.12	0.125	**6.8**	**5.4**
17	0.21	84.9	0.08	0.2	0.5	0.6
18	0.16	83	0.08	0.15	-0.5	-0.5
19	0.162	70	0.049	0.005	-0.4	-0.7
20	0.23	93	0.044	0.11	0.9	1.5
22	0.1	63	0.04	0.1	-1.6	**-2.8**
23	<LOQ	103	-	0.20	(0.3)	-
24	0.13	94	0.04	0.1	-1.1	-1.8
25	<LOQ	101	-	1.6	(**27.8**)	-
26	<0.3	96	-	0.3	(**2.3**)	-
27	-	-	-	-	-	-

The uncertainty value calculated for AFB2 did not meet the criterion $u \leq 0.3\ \sigma_{PT}$, nevertheless, having satisfied the criterion $0.3\ \sigma_{PT} < u \leq 0.7\ \sigma_{PT}$, the σ'_{PT} was calculated. The derived score is reported as z'-score.

Laboratories 6 and 27 used an ELISA method that allows the determination of AFB1 and total aflatoxins, therefore they did not provide a result for AFB2, nor for the recovery factor and the associated uncertainty. Furthermore, 8 laboratories reported the result as <LOQ or less than the value indicated as LOQ, for these, as seen for AFB1, the *proxy* z'-score was calculated. Overall, 13 z'-scores and 8 *proxy* z'-scores could be calculated (reported between brackets in Table 4). In addition to laboratories 2 and 27, those that had a result lower than the LOQ also did not indicate the uncertainty value. In addition, laboratory 9 reported a 0.0 µg/kg for the uncertainty and was not included in the ζ-score calculations; so, it was possible to calculate the ζ-score for 12 laboratories.

Graphically the results, in terms of z'-score and ζ-score, are represented in Figures 8 and 9.

Regarding z'-score, 85% of the laboratories gave a score with satisfactory values ($|z'|\leq 2$) for AFB2, in fact only laboratory 7 had a questionable score ($2<|z'|<3$) and laboratory 15 had an unsatisfactory score ($|z'|\geq 3$). Of the eight laboratories for which the *proxy* z'-score was calculated, three had acceptable values (5, 8, 23). However, in all cases, the LOQ value was above the assigned value, therefore the determination of this level of concentration is out of the concentration range of the applied method for these laboratories.

The ζ-score was calculated for 12 laboratories, 67% of the laboratories gave a score with satisfactory values ($|\zeta|\leq 2$). Laboratories 7 and 22 had a questionable score ($2<|\zeta|<3$) and laboratories 13, 15 and 23 had unsatisfactory scores ($|\zeta|\geq 3$). To be noted that laboratories 7 and 15 had questionable and unsatisfactory scores for both, z- and ζ-score. Laboratory 15 reports a recovery value of 60%, which may be responsible for the overestimation of AFB2, while for laboratory 7, analyzing the information provided by the laboratory, no possible causes for the questionable performance of the method have been identified. For all other cases, as indicated for AFB1, a questionable or unsatisfactory ζ-score value means that the interval defined by the laboratory value and its extended uncertainty ($x_{lab} \pm U_x$) does not include the assigned value.

Laboratories 2, 3 and 14 reported a concentration value for AFB2 lower than the LOQ they indicated for the method, however it was decided to use the data and the z'- and ζ-score values obtained were satisfactory for all 3 participants.

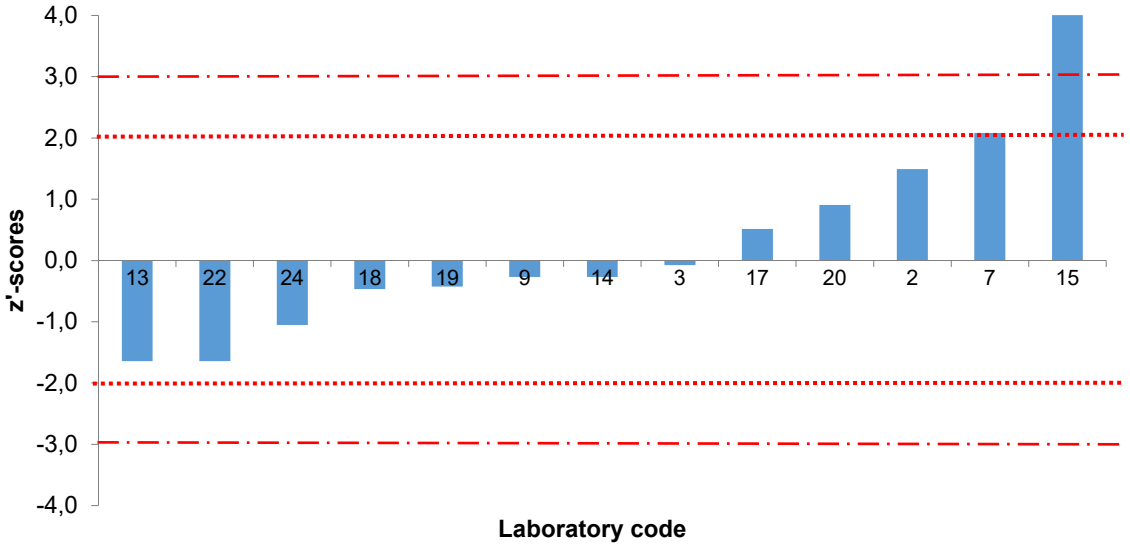

Figure 8. AFB2: z'-score values (assigned value x ± U(x) = 0.18 ± 0.04 µg/kg; σp = 0.05 µg/kg). PT 2025 AFs in pistachios

Figure 9. AFB2: ζ-score values (assigned value x ± U(x) = 0.18 ± 0.04 µg/kg; σp = 0.05 µg/kg). PT 2025 AFs in pistachios

Aflatoxin G1

The analytical results and the participants' z-score and ζ-score values obtained for aflatoxin G1 are summarized in Table 5.

Table 5. AFG1: analytical results and participants *z-score* and ζ-*score* values. PT 2025 AFs in pistachios

Cod. LAB	AFG1 (µg/kg)	R (%)	Ue (µg/kg)	LOQ (µg/kg)	z'-score	ζ-score
2	<LOD	-	-	1.31	(**15.1**)	-
3	0.26	110	0.11	0.2	0.0	0.0
4	<LOQ	-	-	0.5	(**3.5**)	-
5	0.281	103	0.141	0.25	0.3	0.3
6	-	-	-	-	-	-
7	<LOQ	89.3	-	0.25	(-0.1)	-
8	<LOQ	95	-	0.6	(**4.9**)	-
9	-	-	-	0.4	-	-
10	<LOQ	100	-	0.8	(**7.8**)	-
12	<1.25	-	-	1.25	(**14.3**)	-
13	0.205	-	0.031	0.07	-0.8	-1.8
14	0.33	85	0.1	0.3	1.0	1.3
15	0.66	60	0.20	0.5	**5.8**	**3.9**
17	0.25	90.7	0.10	0.2	-0.1	-0.2
18	0.24	81	0.11	0.15	-0.3	-0.3
19	0.254	67	0.076	0.025	-0.1	-0.1
20	0.31	92	0.05	0.12	0.7	1.4
22	0.2	60	0.08	0.1	-0.8	-1.2
23	<LOQ	103	-	0.20	(**-0.8**)	-
24	0.16	95	0.06	0.1	-1.4	**-2.5**
25	Nd	127	-	1.6	(**19.3**)	-
26	<0.6	94	-	0.6	(**4.9**)	-
27	-	-	-	-	-	-

The uncertainty value calculated for AFG1 did not satisfy the criterion $u \leq 0{,}3\ \sigma_{PT}$, nevertheless, having satisfied the criterion $0.3\ \sigma_{PT} < u \leq 0.7\ \sigma_{PT}$, the σ'_{PT} was calculated. The derived score is reported as z'-score.

Laboratories 6 and 27 used an ELISA method that allows the determination of AFB1 and total aflatoxins, therefore they did not provide a result for AFG1, nor for the recovery factor and the associated uncertainty. Furthermore, 8 laboratories reported the result as <LOQ or less than the value indicated as LOQ, for these, as seen for AFB1, the *proxy* z'-score was calculated. In addition, 2 laboratories did not report results for AFG1. Overall, 11 z'-scores and 8 *proxy* z'-scores (reported between brackets in Table 5) could be calculated. All participants who provided a contamination figure for AFG1 accompanied it with the uncertainty value, so it was possible to calculate 11 ζ-scores.

Graphically the results, in terms of z'-score and ζ-score, are represented in Figures 10 and 11.

Regarding z'-score, 91% of the laboratories gave a score with satisfactory values ($|z'| \leq 2$) for AFG1, in fact only laboratory 15 had an unsatisfactory score ($|z'| \geq 3$). Of the eight laboratories for which the z'-score was calculated, only one had a satisfactory value (23). However, in all cases, the LOQ value was above the assigned value, therefore the determination of this level of concentration is out of the concentration range of the applied method for these laboratories.

The ζ-score was calculated for 11 laboratories, 82% of the laboratories gave a score with satisfactory values ($|\zeta| \leq 2$). Laboratory 24 had a questionable score ($2 < |\zeta| < 3$) and laboratories 15, had unsatisfactory scores ($|\zeta| \geq 3$). To be noted that laboratory 15 had unsatisfactory scores for both, z- and ζ-score. As already observed for AFB2, the low recovery value reported for the method may be responsible for the overestimation of AFG1. For all other cases, as indicated for AFB1, a questionable or unsatisfactory ζ-score value means that the interval defined by the laboratory value and its extended uncertainty ($x_{lab} \pm U_x$) does not include the assigned value.

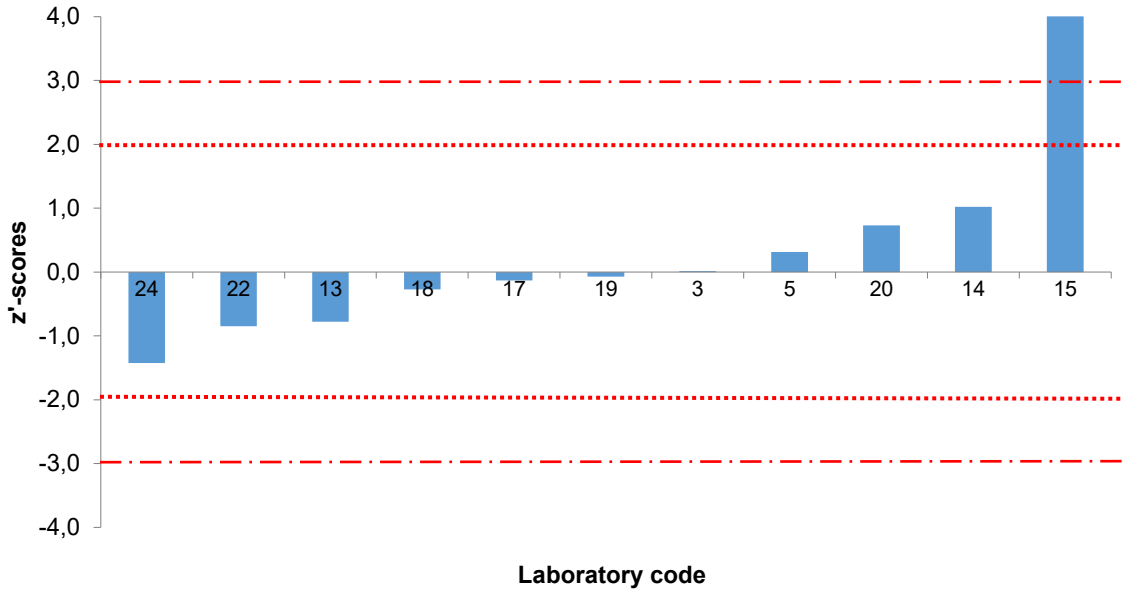

Figure 10. AFG1: z'-score values (assigned value x ± U(x) = 0.26 ± 0.05 µg/kg; σ'p = 0.07 µg/kg). PT 2025 AFs in pistachios

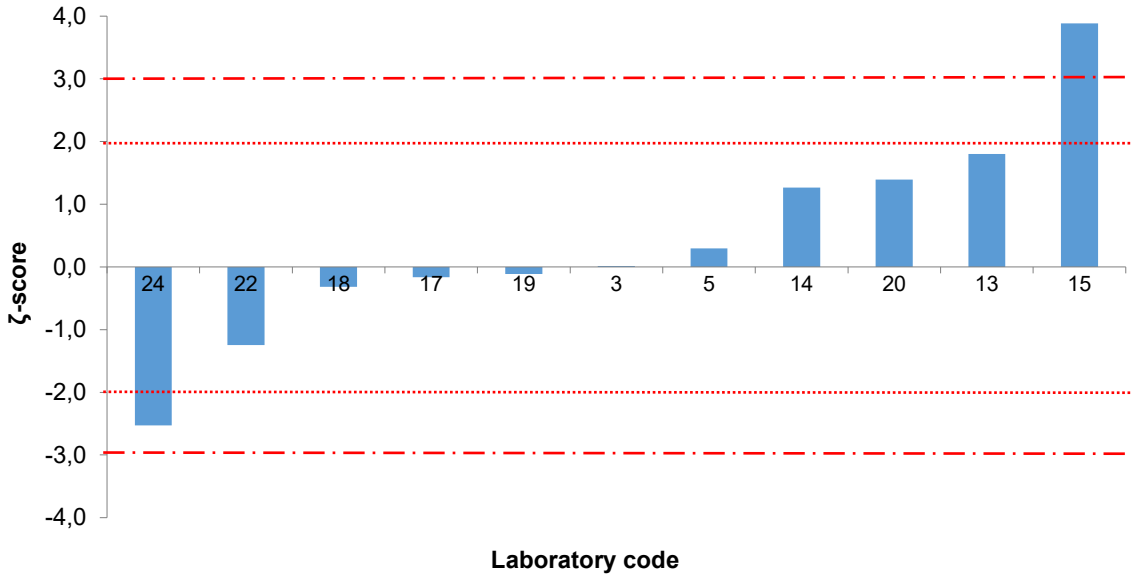

Figure 11. AFG1: ζ-score values (assigned value x ± U(x) = 0.26 ± 0.05 µg/kg; σ'p = 0.07 µg/kg). PT 2025 AFs in pistachios

Aflatoxin G2

The analytical results obtained for aflatoxin B1 are summarized in Table 6.

Table 6. AFG2: analytical results reported. PT 2025 AFs in pistachios

Cod. LAB	AFG2 (µg/kg)	R (%)	Ue (µg/kg)	LOQ (µg/kg)
2	<LOD	-	-	0.39
3	<LOQ	105	-	0.2
4	<LOQ	-	-	0.5
5	<LOQ	98	-	0.25
6	-	-	-	-
7	<LOQ	63.4	-	0.25
8	<LOQ	93	-	0.2
9	-	-	-	0.1
10	<LOQ	68.2	-	0.8
12	0	-	-	1.25
13	0.025	-	0.005	0.1
14	<0.10	67	0.03	0.3
15	0.125	60	0.10	0.125
17	<0.30	92.4	0.03	0.3
18	<0.15	89	-	0.15
19	nn	76	nn	0.026
20	<LOQ	92	-	0.18
22	<0.1	0.64	-	0.1
23	nd	103	-	0.20
24	<LOQ	94	-	0.1
25	nd	90.6	-	1.6
26	<0.3	96	-	0.3
27	-	-	-	-

Since AFG2 was not present in the naturally contaminated sample used for the PT (no assigned value available), z-score and ζ-score were not calculated. However, 2 laboratories gave a concentration value for AFG2.

Laboratory 13 gave a value of 0.025 µg/kg which is lower than the LOQ reported for the method used, while laboratory 15 reported a value equal to the LOQ value of the method. In both cases these results should be considered as false positive.

A false positive is a quantitative result reported by the participant while the toxin is not detected in the PT material by the organizer and/or not detected by most of the other participants. False positives should be interpreted as unsatisfactory performance.

Total aflatoxins

The analytical results and the participants' z-score and ζ-score values obtained for total aflatoxins are summarized in Table 7.

Table 7. AFs: analytical results and participants *z-score* and ζ-*score* values. PT 2025 AFs in pistachios

Cod. LAB	AFs (µg/kg)	R (%)	Ue (µg/kg)	LOQ (µg/kg)	z-score	ζ-score
2	1.89	-	-	-	0.1	-
3	2.38	-	1.05	0.2	1.2	1.0
4	2.2	-	0.8	-	0.8	0.9
5	1.653	-	0.827	-	-0.4	-0.4
6	1.71	111	-	0.1	-0.3	-
7	1.69	-	0.31	-	-0.3	-0.8
8	1.83	-	0.80	0.6	0.0	0.0
9	1.85	120	0.7	0.3	0.0	0.0
10	1.1	-	0.4	0.8	-1.6	**-3.3**
12	not indicated	-	-	-	-	-
13	1.38	-	-	-	-1.0	-
14	2.01	93.5	0.66	0.3	0.4	0.5
15	3.38	60	0.30	0.125	**3.3**	**8.6**
17	2.26	-	-	-	0.9	-
18	1.82	-	0.73	-	0.0	-0.1
19	1.875	70	0.663	0.067	0.1	0.1
20	2.0	92	0.40	0.18	0.3	0.7
22	1.4	-	0.56		-1.0	-1.5
23	not indicated	-	-	0.20	-	-
24	1.44	-	0.31	-	-0.9	**-2.2**
25	<LOQ	-	-	1.6	(-0.5)	-
26	1.90	97	0.57	-	0.1	0.2
27	1.84	85	0.369	1	0.0	0.0

Three laboratories (7, 9, 19) reported a value for total aflatoxins that was different from the sum of the values reported for individual aflatoxins. The values reported by the participant were used in the statistical analyses.

Labs 12 and 23 did not report a value for the sum of aflatoxins, even though they had reported positive results for individual aflatoxins.

As previously, no further processing was considered necessary for the results submitted by participants, so a z-score was not calculated.

Finally, laboratory 25 reported a <LOQ result for AFs which results from also reporting individual aflatoxins as <LOQ. In this case, as seen for AFB1, a *proxy* z'-score was calculated. Four laboratories (2, 7, 13, 17) did not associate the uncertainty value with the concentration provided for AFs. Consistent with the results and information provided, the z-score was calculated for 20 laboratories, and the ζ-score for 16. A *proxy* z-score (reported between brackets in Table 6) was calculated for laboratory 25. Graphically the results, in terms of z-score and ζ-score, are represented in Figures 12 and 13.

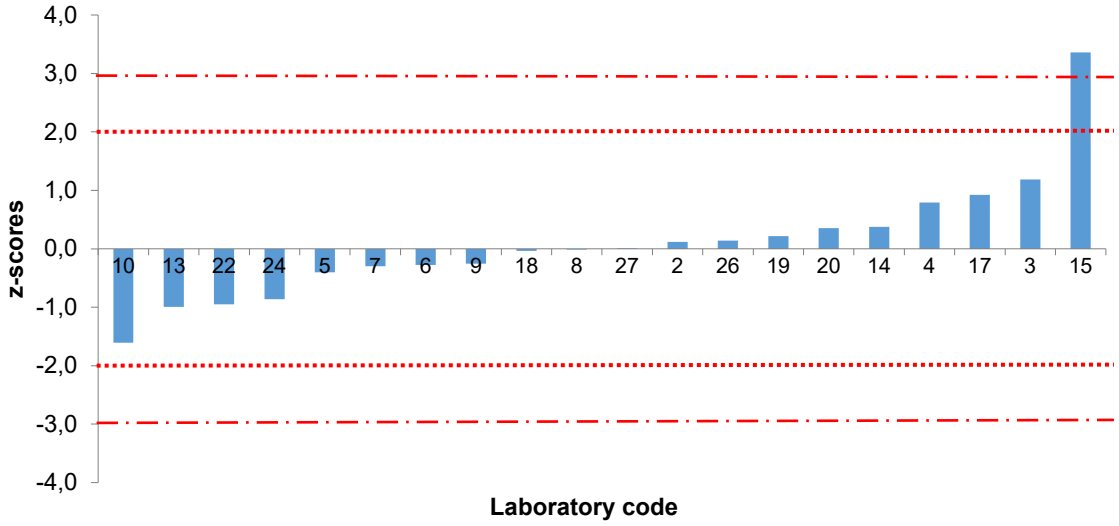

Figure 12. Afs; z-score values (assigned value x ± U(x) = 1.84 ± 0.20 µg/kg; σp = 0.46 µg/kg). PT 2025 AFs in pistachios

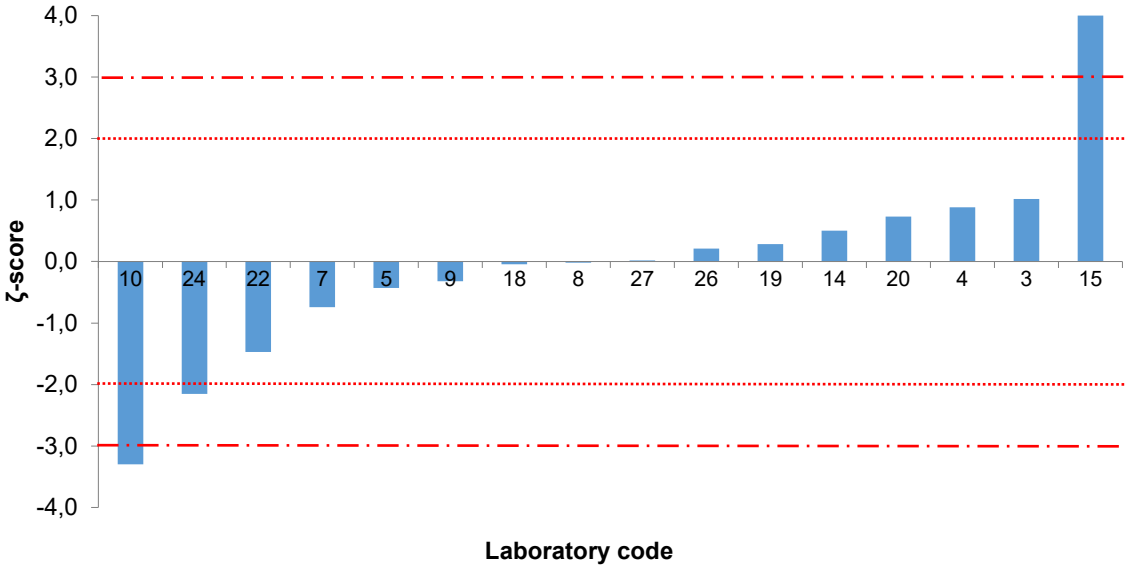

Figure 13. Afs: ζ-score values (assigned value x ± U(x) = 1.84 ± 0.20 µg/kg; σp = 0.46 µg/kg. PT 2025 AFs in pistachios

Regarding z-score, 95% of the laboratories gave a score with satisfactory values ($|z|\leq 2$) for AFs, in fact only laboratory 15 had a questionable score ($2<|z|<3$). The considerations already made regarding the performance of the method used by laboratory 15 also affect the value of the AFs obtained as the sum of the individual aflatoxins. The recovery factor value appears to be too low and therefore responsible for the overestimation incurred by the laboratory. In the EURL-MP guidance document on performance criteria is stated that the average recovery should be between 70 and 120%. This average value, considering the associated relative standard deviation, can also include 60% among the acceptable values. However, in this specific case, the laboratory is advised to verify this recovery value since without correcting the results obtained for the recovery factor, the z- and ζ-scores would all be satisfactory.

The laboratory for which the *proxy* z-score was calculated also obtained acceptable results having provided a LOQ value close to the assigned value. However, in this case, the LOQ value is lower than the assigned value, which therefore should have been quantified by the method.

The ζ-score was calculated for 16 laboratories. Eighty-one% of the laboratories gave a score with satisfactory values ($|\zeta|\leq 2$). Laboratory 24 had a questionable score ($2<|\zeta|<3$), while two laboratories (10 and 15) had unsatisfactory values ($|\zeta|\geq 3$). A questionable or unsatisfactory ζ-score value means that the interval defined by the laboratory value and its extended uncertainty ($x_{lab} \pm U_x$) does not include the assigned value.

CONCLUSIONS

In conclusion, the PT was judged positively both in terms of results and in terms of participation; the successful inclusion in the PT of other European laboratories recruited through the EURL represented a further reason for satisfaction.

The summary of the obtained data for the PT on the determination of aflatoxins in pistachios is shown in Table 8.

Table 8. PT 2025 AFs in pistachios: summary of the obtained data

Analyte	Total number of z-score	Number of |z|<2	Satisfactory results |z| ≤2 (%)	Total number of ζ-score	Number of |ζ|<2	Satisfactory results |ζ| ≤2 (%)
AFB1	21	20	95	20	17	85
AFB2	13	11	85	12	8	67
AFG1	11	10	91	11	9	82
AFs	20	19	95	16	13	81

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APPENDIX A
Documents sent to the participants

A1. PT cover letter

LABORATORIO NAZIONALE DI RIFERIMENTO
MICOTOSSINE E TOSSINE VEGETALI NATURALI

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DEL LAZIO E DELLA TOSCANA
M. ALEANDRI

***Proficiency Testing* for the determination of aflatoxins in pistachios**

Dear Colleague,

thank you very much for your participation in the Proficiency Testing for the analysis of aflatoxins in pistachios.

The parcel shipped contains one pistachios sample of approximately 30 grams. You will receive an e-mail confirming the shipment with attached the acknowledgement of receipt form, the results form and the questionnaire on the applied analytical method.

We ask you to carefully read the following instructions:

- Please fill in the *Acknowledgement of receipt form* and return it upon receipt of the sample by e-mail to dario.lucchetti@izslt and lnr-micotossine-tvn@iss.it
- After arrival the sample should be stored at -18°C
- Before analysis, homogenise the sample according to your laboratory's procedure
- Process the test sample according to your method as a sample for routine analysis. Please note that the homogenization tests have been performed on 15 grams, therefore a test portion of less than 15 grams is not recommended
- Please fill in the *Results form* and the *Questionnaire on the applied analytical method* and send them back by e-mail dario.lucchetti@izslt.it and lnr-micotossine-tvn@iss.it **no later than 30/09/2025**
- Please report the analytical result corrected for recovery in **µg/kg**
- Your laboratory code is _____

Please do not hesitate to contact us in case you have any questions or need any assistance

Kind regards

Italian NRL for Mycotoxins and Plant Toxins, Italian National Institute of Health

Direzione Operativa Chimica, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana

A2. Acknowledgement of receipt

LABORATORIO NAZIONALE DI RIFERIMENTO
MICOTOSSINE E TOSSINE VEGETALI NATURALI

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DEL LAZIO E DELLA TOSCANA
M. ALEANDRI

***Proficiency Testing* for the determination of aflatoxins in pistachios**

ACKNOWLEDGEMENT OF RECEIPT

Please fill in this form and return it upon receipt of the PT material to dario.lucchetti@izslt.it and lnr-micotossine-tvn@iss.it

Laboratory information	
Laboratory	
Reference person	
Laboratory code	
Content of the shipment	
PT letter	
Aknowledgement of receipt	
Results form	
n°1 tube containing 30,00 ± 0,03 g of pistachio*	PTAFs-PIST-____

(*) please indicate the code shown on the samples received

All content is present and was delivered in good condition	YES	NO*
* indicate which content is missing and/or damaged		

Date of receipt _____ Signature _____

A3. Questionnaire on the applied analytical method

Questionnaire on the applied analytical method PT ISS 2025 AFs in pistachios		
General information	Accredited method	YES
	Type of method	CONFIRMATION
	Method reference	
Extraction	Sample weight (g)	
	Extraction solvent composition	
	Amount of extraction solvent (mL)	
	Extraction	SINGLE
	Extraction procedure	ULTRASONIC BATH
	Sample processing	
	Sample clean-up	
HPLC	Injection volume (µL)	
	Mobile phase composition	
	Flow rate (mL/min)	
	Chromatographic column	
	Column temperature (°C)	
	Detector	MS/MS
FLD	λ_{exc}	
	λ_{em}	
MS	Source	ESI
	Precursor ion	
	Product ions, Q/q (m/z)	
Quantification	Calibration	INTERNAL
	Certified standard brand	

A4. Results form

LABORATORIO NAZIONALE DI RIFERIMENTO
MICOTOSSINE E TOSSINE VEGETALI NATURALI

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DEL LAZIO E DELLA TOSCANA
M. ALEANDRI

***Proficiency Testing* for the determination of aflatoxins in pistachios**

RESULT FORM

Laboratory information	
Laboratory	
Reference person	
Laboratory code	

Compound	**Result corrected for recovery (µg/kg)**	**Recovery factor (%)**	**Expanded uncertainty* (µg/kg)**	**LOQ (µg/kg)**
AFB1				
AFB2				
AFG1				
AFG2				
AFs total				

*Coverage factor (k) _____ Confidence level _____

Please fill in this result form and return it back by email to dario.lucchetti@izslt.it and lnr-micotossine-tvn@iss.it **no later than 30/09/2025**

Signature _____

APPENDIX B
List of the participants

The order of indication shown in the table does not correspond to the assigned ID code.

Name of the laboratory	Reference person	City/Country
AGROLAB LUFA	Kirch Katarina	Kiel/Germany
ARPA Campania	Aquila Maria Grazia	Napoli/Italy
ARPA Friuli Venezia Giulia	Falomo Jari	Palmanova (UD)/Italy
ARPA Liguria	Ferro Marta	Genova/Italy
ATS Milano	Amato Giuseppina	Milano/Italy
Central Laboratory for Chemical Testing and Control Bulgarian Food Safety Agency	Temelcheva Elka	Sofia/Bulgaria
Croatian National Institute of Public Health	Pukljak Ivana	Zagreb/Croatia
CVUA	Baumann Sarah	Stuttgart/Germany
Eurofins WEJ Contaminants GmbH	Reeber Tanja	Hamburg/Germany
Institut für Lebensmittelchemie	Arnold Lukas	Trier/Germany
IZS Lombardia ed Emilia Romagna	Caprai Elisabetta	Bologna/Italy
IZS del Mezzogiorno	Buonocore Emanuela	Portici (NA)/Italy
IZS Puglia e Basilicata	Iammarino Marco	Foggia/Italy
IZS Sicilia	Macaluso Andrea	Palermo/Italy
IZS delle Venezie	Contiero Lidia	Padova/Italy
Kantonales Laboratorium	Stoth Frederike	Bern/Switzerland
Kantonales Laboratorium Thurgau	Streb Sebastian	Frauenfeld/Switzerland
Laboratoire Vétérinaire et Alimentaire	Schaeffer Charline	Dudelange/Luxembourg
Norwegian Veterinary Institute	Tukun Feng Ling	Oslo/Norway
Sanitary Veterinary and Food Safety Laboratory	Negreanu Cătălin	Bucharest/Romania
Sample Control d.o.o	Maric Leo	Zagabria/Croatia
State Laboratory (Health Section)	Bermingham Sinead	Celbridge/Ireland
State Veterinary and Food Institute	Hudakova Simona	Košice/Slovakia
Swidish Veterinary Agency	Roussos De Kock	Stockholm/Sweden
Teaching Institute of Public Health	Klaric Sanja	Rijeka/Croatia

APPENDIX C
Technical information on the analytical method applied by the participants

Table C1. Technical information on the analytical methods used by the participants. Sample preparation

Technical information	Laboratory Code ID
Accredited method	
Yes	3, 4, 5, 6, 8, 9, 10, 12, 13, 14, 17, 18, 19, 22, 23, 24, 26, 27
No	15
Type of method	
Screening	6
Confirmatory	3, 4, 5, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 26, 27
Sample weight, g	
≤5	3, 5, 6, 12, 14, 19, 22
>5	4, 8, 9, 10, 13, 15, 17, 18, 23, 24, 26, 27
Extraction solvent composition	
MeOH:H_2O	5, 6,10, 14, 18, 19, 24, 27
MeOH:H_2O+ hexane/heptane	8, 9/22
ACN:CH_3COOH	3, 15
ACN:H_2O:CH_3COOH (79:20:1)	17
ACN:H_2O (60:40)	4, 12, 13, 23
H_2O (100%)	12
Extraction	
Single	3, 4, 6, 8, 9, 10, 13, 14, 15, 17, 18, 19, 22, 23, 24, 26, 27
Multiple	5, 12
Extraction procedure	
Blender	4, 5, 24, 26
Shaker	3, 6, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19, 22, 23, 27
Sample processing	
Dilution	4, 6, 8, 10, 12, 13, 17, 19, 23, 26
Filtration	6, 8, 10, 18, 19, 22, 24, 27
Centrifugation	3, 5, 12, 13
No processing	15
Not specified	9, 14
Sample clean-up	
IAC	2, 4, 5, 8, 9, 10, 14, 18, 22 ,23, 24, 26
QuEChERS	3
SPE	13
None	6, 12, 15, 17

Table C2. Technical information on the analytical methods used by the participants. Sample determination

Technical information	Laboratory Code ID
ELISA	
	6, 27
HPLC	
	3, 4, 5, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 26
V_{INJ}, µL	
≤ 10	3, 4, 5, 12, 13, 15, 18, 19, 23
≥ 30	8, 9, 10, 14, 17, 22, 24, 26
Mobile phase composition	
H_2O $HCOONH_4$ HCOOH; MeOH	13
H_2O $HCOONH_4$ HCOOH; MeOH HCOOH	3
H_2O $HCOONH_4$ HCOOH; MeOH $HCOONH_4$ HCOOH	5
H_2O MeOH $HCOONH_4$ HCOOH; MeOH H_2O $HCOONH_4$ HCOOH	23
H_2O MeOH CH_3COONH_4 CH_3COOH; MeOH CH_3COONH_4 CH_3COOH	15
H_2O CH_3COONH_4 CH_3COOH HCOOH; MeOH CH_3COONH_4 HCOOH	19
MeOH $HCOONH_4$ HCOOH	18
H_2O ACN HCOOH; ACN HCOOH	4
H_2O MeOH ACN	8, 9, 10, 24, 26
H_2O MeOH ACN KBr HNO_3	14
H_2O MeOH ACN H_3PO_4	22
H_2O CH_3COOH NH_4F; ACN CH_3COOH NH_4F	12
H_2O MeOH	17
Flow rate. mL/min	
≤ 0,35	4, 13, 15, 18, 23
≥ 0,4	3, 5, 8, 9, 10, 12, 14, 17, 19, 22, 24, 26
LC column packing	
C18	3, 4, 5, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 26
Column temperature. °C	
Not specified	10
25-30	8, 13, 14, 24, 26
40-50	3, 4, 5, 9, 12, 15, 17, 18, 19, 22, 23
Detector	
FLD	8,9,10,14,22,24,26
λ_{exc}=365 nm ; λ_{em}=435 nm	8, 10
λ_{exc}=362 nm ; λ_{em}=440 nm	14, 24
λ_{exc}=366 nm ; λ_{em}=435 nm	26
Not specified	9, 22
MS/MS	3, 4, 5, 6, 12, 13, 15, 17, 18, 19, 23
Source	
ESI	3, 4, 5, 6, 12, 13, 15, 17, 18, 19, 23

Technical information	Laboratory Code ID
AFB1	
Precursor ion	
313	3, 4, 5, 12, 13, 15, 19, 23
Product ion	
213	12
241	3, 4, 5, 13, 15, 19, 23
269	3, 4, 5, 15
285	3, 13, 19, 23
341	12
AFB2	
Precursor ion	
315	3, 4, 12, 15, 23
332	19
Product ion	
243	3, 15
259	3, 5, 12, 15, 19, 23
271	15
287	3, 5, 12, 19, 23
AFG1	
Precursor ion	
329	3, 5, 12, 15, 19, 23
Product ion	
199	3, 15
200	3, 5, 12, 15
243	3, 5, 12, 15, 19, 23
311	19, 23
AFG2	
Precursor ion	
331	3, 5, 12, 15, 19, 23
Product ion	
57	15
95	15
189	12
217	3
239	15
245	3, 5, 12, 19, 23
285	5
313	3, 19, 23
Calibration	
Internal	3, 4, 6, 9, 12, 17, 18, 19, 22, 23
External	5, 8, 10, 13, 14, 15, 24, 26, 27

Serie Rapporti ISTISAN
numero di maggio 2026

Stampato in proprio
Servizio Comunicazione Scientifica – Istituto Superiore di Sanità

Roma, giugno 2026