# PROFICIENCY TEST FINAL REPORT

## Determination of ochratoxin A in green coffee by immunoaffinity column clean-up and LC/TLC.

**Ministry of Agriculture, Livestock and Supply**

**Laboratory for Quality Control and Food Safety – LACQSA**

**LANAGRO-MG**

**Belo Horizonte**

**Brazil, 2006**

Implementation of the Objective 4

Global FAO/ICO/CFC Project GCP/INT/743/CFC - ENHANCEMENT OF COFFEE QUALITY THROUGH PREVENTION OF MOULD FORMATION

(STUDY PLAN - PE LACQSA 035)

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**Abstract**

A Proficiency Test has been performed under the sponsorship of Project “ENHANCEMENT OF COFFEE QUALITY THROUGH PREVENTION OF MOULD FORMATION”. The project was comprised of: a) the preparation of reference material (OTA standard solution and OTA naturally contaminated green coffee samples) and b) one pre-proficiency test and five proficiency test rounds. The project has involved ten laboratories from seven different coffee producing countries as follows: Brazil, Colombia, Uganda, Kenya, Cote D´Ivoire, Indonesia, India and France. The OTA naturally contaminated green coffee reference materials were prepared by: grinding, sieving, homogenizing and OTA analysis. The homogeneous batches were packed under vacuum in aluminium foil sachets, labelled and coded. The homogeneity of the reference material, in the bulk and after packing, was analysed by one-way analysis of variances (ANOVA) according to the International Harmonized Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories and as established by ISO 43-1, at 95% of confidence level by calculating an F-statistic and $Ss/\sigma$ ($\sigma$=15%). Seven homogeneous green coffee materials were prepared: one blank and six naturally contaminated samples. In the five proficiency test rounds, the ten participating laboratories were sent, by express delivery, a refrigerated parcel containing the following items: coded arabica green coffee naturally contaminated samples, a blank arabica green coffee sample for spiking, flask containing ochratoxin A working standard solution for calibration purpose, flask of blind ochratoxin A working standard solution for spiking purpose, documentation comprising of: additional instructions, test material receipt form, results reporting sheets, analytical work questionnaire and the method protocol containing a flow chart of the OTA analysis. The laboratories were required to analyse the arabica green coffee reference samples on the same day, in duplicate, following exactly the method protocol and the additional instructions. The use of OTA standard solution prepared by LACQSA guaranteed that all laboratories used trustful and traceable standard solutions during the OTA analysis. The results obtained in the five proficiency rounds were evaluated by the z-score function and repeatability/reproducibility were also calculated. A report discussing the results and problems reported by the laboratories during each of the proficiency rounds was elaborated with comments to help the laboratories to improve their analytical procedures and quality assurance systems. With the organization and implementation of the proficiency testing program a net of laboratories for OTA analysis in the coffee producing countries were build up and technicians from different coffee institutions were trained to analyse OTA in coffee using both HPLC and TLC quantification. Technical difficulties in some laboratories concerning equipments and skilled technicians exist yet, and need to be solved but the initial laboratory facilities are in place

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# PROFICIENCY TESTING – FINAL REPORT

## Determination of ochratoxin A in green coffee by immunoaffinity column clean-up and LC/TLC

### 1 Introduction

Proficiency Testing by interlaboratory comparisons is used to determine the performance of individual laboratories for specific tests or measurements, and to monitor the continuing performance of laboratories. Participation in proficiency testing schemes provides laboratories with an objective means of assessing and demonstrating the a reliability of the data they are producing, where one of the main uses of proficiency testing schemes is to asses laboratories ability to perform tests competently [01]. Statically, the majority of results will be centred on a mean value, with some results lied at the extremes of the distribution. The statistics of a normal distribution mean that 95% of data points will lie between a z-score of –2 and +2 [02].

A **Proficiency Testing Program** was performed under the sponsorship of Global FAO/ICO/CFC Project GCP/INT/743/CFC "ENHANCEMENT OF COFFEE QUALITY THROUGH PREVENTION OF MOULD FORMATION" and LACQSA coordination. The project comprised of the preparation of reference material (OTA standard and OTA naturally contaminated samples), one pre-proficiency test and 05 proficiency test rounds

The project involved partners in Brazil, Colombia, Uganda, Kenya, Cote D´Ivoire, Indonesia, India and France.

Project partners:

- Eugênia Azevedo Vargas, Eliene Alves dos Santos, Luciana de Castro, Regina Coeli Alves França, Cristiane Maria Gomes da Silva, Thais Alves de Sá - LACQSA-MG. MAPA. Brazil
- Otniel Freitas-Silva - EMBRAPA/CTAA. Brazil
- Cahya Ismayadi - Indonesian Coffee and Cocoa Research Institute (ICCRI). Indonesia
- Gloria Ines Puerta Quintero - Centro Nacional de Investigaciones de Café/Federación Nacional de Cafeteros de Colombia (Cenicafe). Colombia
- Joseph Karanja Mburu - Kenyan Coffee Research Foundation (KCRF). Kenya
- Kakuba Albertina - Uganda Coffee Development Authority (UCDA). Uganda
- Louis Ban Koffi - CNRA/SRT. Cote D´Ìvoire
- Y Raghuramulu - Indian Coffee Board of India. India
- Bernard Guillot - CIRAD. France

Additional participant

- Hissae Ida - SGS Santos. Brazil

## 2 Objectives

The objectives of the proficiency tests were to:

✓ Evaluate the performance of the laboratories and the main difficulties encountered in performing the analytical procedure for determination of ochratoxin A (OTA) in green coffee;

✓ Contribute to the harmonisation of analytical procedures of the partner laboratories;

✓ Contribute to the laboratory's proficiency in OTA analysis.

## 3 Material

### 3.1 Matrix

- Arabica green coffee

### 3.2 Reference materials

#### 3.2.1 Ochratoxin A standard solution

Ochratoxin A (Sigma ™) standard solutions were prepared, standardized in toluene-acetic acid (99+1, v/v) [3, 4, 5] and envased in ampoules. The ampoules were sent as blind ochratoxin A standard solution for spiking purpose and as OTA standard solution for calibration curve (1 µg/mL).

#### 3.2.2 LACQSA arabica green coffee reference materials

A total of seven naturally contaminated samples with undisclosed contamination and a blank sample for spiking purpose were prepared by LACQSA according to ISO GUIDE 043 [6] and IUPAC [7, 8]

The concentration and the standard deviation determined for the seven batches of reference material are given in **Table 1.**

## 4 Methods

### 4.1 Test material – Preparation and homogenisation

Approximately 10-30 kg of OTA naturally contaminated green coffee samples, were prepared by LACQSA by grinding and sieving the green coffee beans through 0.5 - 1.0 mm screens (80% of the material should be less than 0.5 mm particle size) and thoroughly homogenized for at least 6 hours in 30 min cycles. After each 30 minutes cycle, the batches were mixed using a spade and hands.

Ten samples of 50 g were randomly taken from each bulk material and analysed, in duplicate, to check the homogeneity. The final homogeneous batches of materials were packed under vacuum

in aluminium foil sachets (~50g), labelled and coded. During the packing, the first sachet and one sachet of every 10$^{th}$ sachet were taken out from the filling sequence for final homogeneity testing, were analysed, in duplicate, using the OTA Standard Operation Procedure described in the proficiency protocol.

The homogeneity of the reference material, in the bulk and after packing, was analysed by one-way analysis of variances (ANOVA) according to International Harmonized Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories [8] as established by ISO 43-1 [6], at 95% of confidence level by calculating an F-statistic and $S_s/\sigma$ ($S_s/\sigma$=15%). A stricter $\sigma$ of 15% instead of 22% [9] was employed to evaluate the homogeneity of the test materials.

All batches of test material, prior to and after packaging, were stored under – 18 °C and protected from light.

No significant difference could be observed for within - and between-sachets standard deviation, neither by F-test (ANOVA) (F-calculated < F-critical at 5%) nor by Ss/$\sigma$ for any contaminated material (6, 7, 8).

The mean values for ochratoxin A contents determined during the final homogeneity study in the packed naturally contaminated green coffee materials were considered as assigned values for the batches, and the range of acceptability for each material was considered as:

- The mean ± one standard deviation: ($\overline{X}$ ± s);
- The mean ± two standard deviation ($\overline{X}$ ± 2s).

**Table 1:** Assigned value and standard deviation to each reference material

| Sample | OTA assigned value (ng/g) | s (ng/g) | 2s (ng/g) |
|---|---|---|---|
| **A** | 1.34 | 0.14 | 0.27 |
| **B** | 2.44 | 0.43 | 0.86 |
| **C** | 4.28 | 0.28 | 0.56 |
| **D** | 5.15 | 0.77 | 1.54 |
| **E** | 5.40 | 0.52 | 1.04 |
| **F** | 13.46 | 1.18 | 2.36 |
| **G** | ND (<0.12) | - | - |

## 4.2 Distribution

Ten (10) participating laboratories were sent by express delivery a refrigerated parcel containing the following items:

a. Coded arabica green coffee naturally contaminated samples
b. Blank arabica green coffee sample (< 0.12 ng/g) for spiking
c. A flask of ochratoxin A working standard solution of 1.0 µg/mL for calibration purpose
d. A flask of blind ochratoxin A working standard solution for spiking purpose
e. Additional instructions for the participants
f. A Test Material Receipt Form
g. Results reporting sheets for analytical data as well as criticisms and suggestions
h. Analytical Work Questionnaire
i. Flow chart of OTA analysis

Participants were required to return each round test material receipt form as soon as they got the parcels and return the results to LACQSA on specified date.

## 4.3 Analysis

The laboratories were required to analyse the arabica green coffee reference samples on the same day, in duplicate, following exactly the method protocol and the additional instructions to the participants for the five rounds.

Laboratories were asked to spike the blank materials with 150 µL of the blind ochratoxin A standard solution, in all rounds. As the OTA concentration in the blind standard solution was 1.0 µg/mL the spiked sample level was 6.00 ng/g.

## 4.4 Evaluation of the results

The results were evaluated by LACQSA (replicate) and displayed in bar graphs (precision and accuracy) taking in consideration $\bar{x} \pm s$ and $\bar{x} \pm 2s$, in which:

$\bar{x}$ is the value that best represents the true measure of ochratoxin A (assigned value) in the reference sample (as per evaluation in the homogeneity tests) [6, 7, 8];

s is the standard deviation (as per evaluation in the homogeneity tests) [6, 7, 8];

The variation of 22% [9] was used to evaluate the variability between replicate analysis. Results were considered acceptable if within the interval of $\bar{x} \pm 2s$.

The results were also displayed in control graphs using z-score function being calculated by the following equation:

$$z = \frac{x - \bar{x}}{\sigma}$$

In which:

$x$ is the contamination value determined by the Laboratory;

$\bar{x}$ is the value that best represents the true measure of ochratoxin A (assigned value) in the reference sample (as per evaluation in the homogeneity tests);

$\sigma$ is the standard deviation of the value that best represents the true measure of ochratoxin A. being the standard deviation ( $\sigma$ ) calculated as b $\bar{x}$ .

where:

b= % $RSD_R$ /100

For concentrations of the analyte <120 ng/g, relative standard deviation ($RSD_R$) is obtained from modified Horwitz's equation, where $RSD_R$ = 22% [9].

The z-score interpretation is made as described below:

| Ranges | The Laboratory result is considered: |
|---|---|
| **If $\|z\| \leq 2$** | **"Satisfactory"** |
| **If $2 < \|z\| \leq 3$** | **"Questionable"** |
| **If $\|z\| > 3$** | **"Unsatisfactory"** |

The recovery results were deemed as acceptable if complying with CEN (1999) [10] i.e recoveries ranging from 70-110 % for a spiked level in the range of 1-10 ng/g. Considering the recovery range of 70-110% for the spiked level of 6.00 ng/g, used in all Proficiency Rounds, the acceptable range was 4.20 to 6.60 ng/g.

## 5 Results

Each participant was given a Laboratory number in order to maintain the confidentiality.

### 5.1 Analytical results

The laboratories results are given in Table 2 to 8 (annex) and displayed in Graphs 1 to 17. As Laboratory 02 returned the results only for one Proficiency Round, there were no Graphs for that Laboratory.

The results were not corrected for the recovery of OTA in the spiked sample.

**Table 2 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference materials – Samples A ( 1.34 ng/g) and C ( 4.28 ng/g).

| Laboratory number | Sample A ( 1.34ng/g) | | | | | | Sample C ( 4.28 ng/g). | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fourth round | | | Fifth round | | | Fourth round | | | Fifth round | | |
| | Replicate 1 | Replicate 2 | Mean | Replicate 1 | Replicate 2 | Mean | Replicate 1 | Replicate 2 | Mean | Replicate 1 | Replicate 2 | Mean |
| **1** | 2.05 | 1.64 | 1.85 | 4.62 | 1.60 | 3.11 | 3.90 | 3.98 | 3.94 | 4.53 | 18.13 | 11.33 |
| **2** | 1.84 | ** | NI | - | - | - | 1.95 | ** | NI | - | - | - |
| **3** | 2.46 | ** | NI | 1.56 | 1.20 | 1.38 | 2.04 | 2.10 | 2.07 | 4.91 | 3.11 | 4.01 |
| **4** | 5.12 | 5.40 | 5.26 | 1.43 | 1.41 | 1.42 | 2.86 | 2.97 | 2.92 | 3.80 | 3.99 | 3.90 |
| **5** | 1.246 | 1.263 | 1.25 | 1.24 | 1.31 | 1.28 | 1.794 | 1.481 | 1.64 | 1.38 | 1.40 | 1.39 |
| **6** | 1.26 | 1.15 | 1.21 | 2.07 | 1.38 | 1.73 | 3.27 | 3.25 | 3.26 | 4.85 | 6.15 | 5.50 |
| **7** | * | * | * | 1.61 | 1.57 | 1.59 | 4.24 | 4.33 | 4.29 | 4.51 | 4.75 | 4.63 |
| **8** | - | - | - | - | - | - | - | - | - | - | - | - |
| **9** | 1.61 | 1.67 | 1.64 | 1.89 | 1.88 | 1.89 | 3.79 | 3.68 | 3.74 | 3.50 | 3.02 | 3.26 |
| **10** | 1 | 2 | 1.5 | - | - | - | 6 | 5 | 5.5 | - | - | - |

[1]Data not corrected for recovery, *Laboratory reported analytical problem **–** No results were sent by the laboratory **Single analysis; NI: no information

**Note 1:** Results are shown as reported by the laboratories

**Table 3 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference material - Sample B (2.44 ng/g).

| Laboratory number | First round | | | Second round | | | Third round | | | Fourth round | | | Fifth round | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** |
| **1** | 3.94 | 4.30 | 4.12 | 2.48 | 2.84 | 2.66 | 3.48 | 3.84 | 3.66 | 2.74 | 5.95 | 4.35 | 3.85 | 3.02 | 3.44 |
| **2** | - | - | - | - | - | - | 2.82 | ** | NI | 1.46 | ** | NI | - | - | - |
| **3** | 3.25 | 2.76 | 3.01 | 3.06 | 2.06 | 2.56 | 3.20 | 2.17 | 2.69 | * | * | * | 2.29 | 2.43 | 2.36 |
| **4** | 1.87 | 2.07 | 1.97 | 2.51 | 2.65 | 2.58 | 1.83 | 1.77 | 1.80 | 2.39 | 1.88 | 2.14 | 2.72 | 2.56 | 2.64 |
| **5** | 3.95 | 2.94 | 3.45 | 2.665 | 2.362 | 2.51 | 10.56 | 9.41 | 9.99 | 0.882 | 0.930 | 0.91 | 2.68 | 2.42 | 2.55 |
| **6** | 2.79 | 2.45 | 2.62 | 3.35 | 2.19 | 2.77 | 2.80 | 2.46 | 2.63 | 2.87 | 2.91 | 2.89 | 2.72 | 3.59 | 3.16 |
| **7** | 2.50 | 2.49 | 2.50 | 2.61 | 2.72 | 2.67 | 2.84 | 2.26 | 2.55 | 4.17 | 3.15 | 3.66 | 2.87 | 3.12 | 3.00 |
| **8** | 5.0 | 3.0 | 4.00 | 1 | 1 | 1.00 | 1.00 | 1.00 | 1.00 | - | - | - | - | - | - |
| **9** | 2.85 | 2.78 | 2.82 | 2.84 | 2.99 | 2.92 | 2.30 | 2.51 | 2.41 | 2.54 | 2.30 | 2.42 | 1.23 | 1.02 | 1.13 |
| **10** | 8 | 6 | 7.00 | 2 | 2 | 2.00 | 2 | 2 | 2.00 | 6 | 6 | 6.00 | - | - | - |

[1]Data not corrected for recovery, *Laboratory reported analytical problem **–** No results were sent by the laboratory **Single analysis; NI: no information.

**Note 1:** Results are shown as reported by the laboratories

**Table 4 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference material - Sample D (5.15 ng/g).

| Laboratory number | First round | | | Second round | | | Third round | | | Fourth round | | | Fifth round | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** |
| **1** | 7.35 | * | 7.35 | 6.49 | 6.78 | 6.64 | 8.42 | 7.72 | 8.07 | 6.85 | 7.48 | 7.17 | 9.29 | 6.77 | 8.03 |
| **2** | - | - | - | - | - | - | 2.48 | ** | NI | 2.51 | ** | ** | - | - | - |
| **3** | 7.19 | 6.39 | 6.79 | 4.59 | 3.55 | 4.07 | 4.00 | 3.96 | 3.98 | 2.74 | 2.43 | 2.59 | 3.80 | 3.33 | 3.57 |
| **4** | 5.72 | 4.08 | 4.90 | 5.15 | 3.99 | 4.57 | 4.91 | 4.77 | 4.84 | 4.57 | 5.08 | 4.83 | 7.34 | 8.72 | 8.03 |
| **5** | 2.39 | 3.19 | 2.79 | 4.398 | 5.676 | 5.04 | 17.56 | 15.08 | 16.32 | 1.564 | 1.957 | 1.76 | 3.14 | 3.09 | 3.12 |
| **6** | 6.06 | 5.21 | 5.64 | 4.95 | 4.58 | 4.77 | 5.60 | 5.89 | 5.75 | 5.16 | 4.19 | 4.68 | 7.70 | 7.46 | 7.58 |
| **7** | 4.68 | 5.25 | 4.97 | 4.28 | 4.75 | 4.52 | 6.59 | 5.92 | 6.26 | 6.17 | 5.18 | 5.68 | 4.69 | 5.66 | 5.18 |
| **8** | 4 | 3.1 | 3.55 | 4 | 4 | 4.00 | nd | nd | nd | - | - | - | - | - | - |
| **9** | 5.68 | 5.57 | 5.63 | 7.06 | 6.96 | 7.01 | 5.13 | 5.96 | 5.55 | 5.21 | 5.35 | 5.28 | 4.39 | 4.36 | 4.38 |
| **10** | 2 | 1 | 1.50 | 1.50 | 1 | 1.25 | 6 | 5 | 5.50 | 7 | 9 | 8.00 | - | - | - |

[1]Data not corrected for recovery; *Laboratory reported analytical problem; **–** No results were sent by the laboratory**;** **Single analysis; nd: not detected; NI: no information

**Note 1:** Results are shown as reported by the laboratories

**Table 5 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference material - Sample E (5.40 ng/g).

| Laboratory number | Second round | | | Third round | | | Fourth round | | | Fifth round | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** |
| **1** | 11.44 | 9.54 | 10.49 | 9.88 | 7.76 | 8.82 | 8.61 | 7.72 | 8.17 | 9.27 | 4.69 | 6.98 |
| **2** | - | - | - | nd | ** | NI | 1.93 | ** | NI | - | - | - |
| **3** | 5.27 | 5.53 | 5.40 | 6.69 | 4.75 | 5.72 | 2.98 | 1.81 | 2.40 | 3.78 | 5.57 | 4.68 |
| **4** | 5.68 | 4.96 | 5.32 | 5.27 | 6.36 | 5.82 | 1.74 | 1.79 | 1.77 | 6.55 | 5.85 | 6.20 |
| **5** | 3.321 | 2.277 | 2.80 | 2.45 | 3.29 | 2.87 | 1.849 | 1.826 | 1.84 | 0.67 | 0.79 | 0.73 |
| **6** | 5.82 | 6.60 | 6.21 | 5.57 | 5.67 | 5.62 | 5.60 | 5.52 | 5.56 | 6.90 | 7.88 | 7.39 |
| **7** | 5.67 | 6.14 | 5.91 | 7.08 | 6.99 | 7.04 | 6.26 | 5.86 | 6.06 | 6.41 | 6.19 | 6.30 |
| **8** | 7 | 4 | 5.50 | 4.00 | 4.00 | 4.00 | - | - | - | - | - | - |
| **9** | 6.84 | 6.95 | 6.90 | 7.43 | 7.24 | 7.34 | 7.51 | 7.59 | 7.55 | 9.43 | 9.16 | 9.30 |
| **10** | 0.5 | 0.5 | 0.50 | 7 | 7 | 7.00 | 4 | 5 | 4.50 | - | - | - |

[1]Data not corrected by recovery; *Laboratory reported analytical problem; **–** No results were send by the laboratory; **Single analysis; nd: not detected; NI: no information.

**Note 1:** Results are shown as reported by the laboratories

**Table 6 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference material - Sample F (13.46 ng/g).

| Laboratory number | First round | | | Second round | | | Third round | | |
|---|---|---|---|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** | Replicate 1 | Replicate 2 | **Mean** |
| **1** | 22.44 | * | * | 24.80 | 20.31 | 22.56 | 18.73 | 22.70 | 20.72 |
| **2** | - | - | - | - | - | - | 7.62 | ** | NI |
| **3** | 15.38 | 17.11 | 16.25 | 10.49 | 16.03 | 13.26 | 11.31 | 11.91 | 11.61 |
| **4** | 8.23 | 8.26 | 8.25 | 14.27 | 11.12 | 12.70 | 11.06 | 9.95 | 10.51 |
| **5** | 7.94 | 10.08 | 9.01 | 5.719 | 6.194 | 5.96 | 12.88 | 11.96 | 12.42 |
| **6** | 12.4 | 16.89 | 14.65 | 14.61 | 15.44 | 15.03 | 17.15 | 16.82 | 16.99 |
| **7** | 12.67 | 12.26 | 12.47 | 11.34 | 11.10 | 11.22 | 12.74 | 12.77 | 12.76 |
| **8** | 10.4 | 12.0 | 11.20 | 7 | 7 | 7.00 | 6.00 | 6.00 | 6.00 |
| **9** | 18.96 | 17.28 | 18.12 | 13.40 | 14.75 | 14.08 | 12.70 | 12.94 | 12.82 |
| **10** | 10 | 10 | 10 | 4 | 4 | 4.00 | 10 | 10 | 10.00 |

[1]Data not corrected by recovery; *Laboratory reported analytical problem; **–** No results were sent by the laboratory; **Single analysis; nd: not detected;

NI: no information.

**Note 1:** Results are shown as reported by the laboratories

**Table 7 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference material - Sample G (<0.12 ng/g).

| Laboratory number | First round | | Second round | | Third round | | Fourth round | | Fifth round | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 |
| **1** | 0.18 | * | * | 0.04 | 0.91 | 0.86 | 0.30 | 0.30 | 0.31 | 0.23 |
| **2** | - | - | - | - | 3.81 | ** | 2.30 | ** | - | - |
| **3** | 0.91 | 0.72 | nd | nd | nd | nd | nd | nd | 0.14 | 0.07 |
| **4** | nd | nd | <0,05 | <0,05 | < 0,05 | < 0,05 | < 0,05 | < 0,05 | <0.05 | <0.05 |
| **5** | 0.6 | 0.48 | 0.122 | 0.389 | 0.46 | 0.09 | 0.296 | 0.246 | 0.23 | 0.25 |
| **6** | 0.27 | 0.00 | 0.05 | 0.16 | 0.09 | 0.40 | - | - | 0.27 | 0.24 |
| **7** | nd | nd | nd | nd | nd | nd | * | * | nd | nd |
| **8** | 1.5 | 1.5 | 3 | 3 | 2.67 | 2.67 | - | - | - | - |
| **9** | <0,1 | <0,1 | ND | ND | < 0,1 | < 0,1 | < 0,1 | < 0,1 | <0.1 | <0.1 |
| **10** | 2 | 3 | 0.75 | 0.5 | 0 | 0 | 0 | 0 | - | - |

[1]Data not corrected by recovery; *Laboratory reported analytical problem; **–** No results were sent by the laboratory; **Single analysis; nd: not detected.

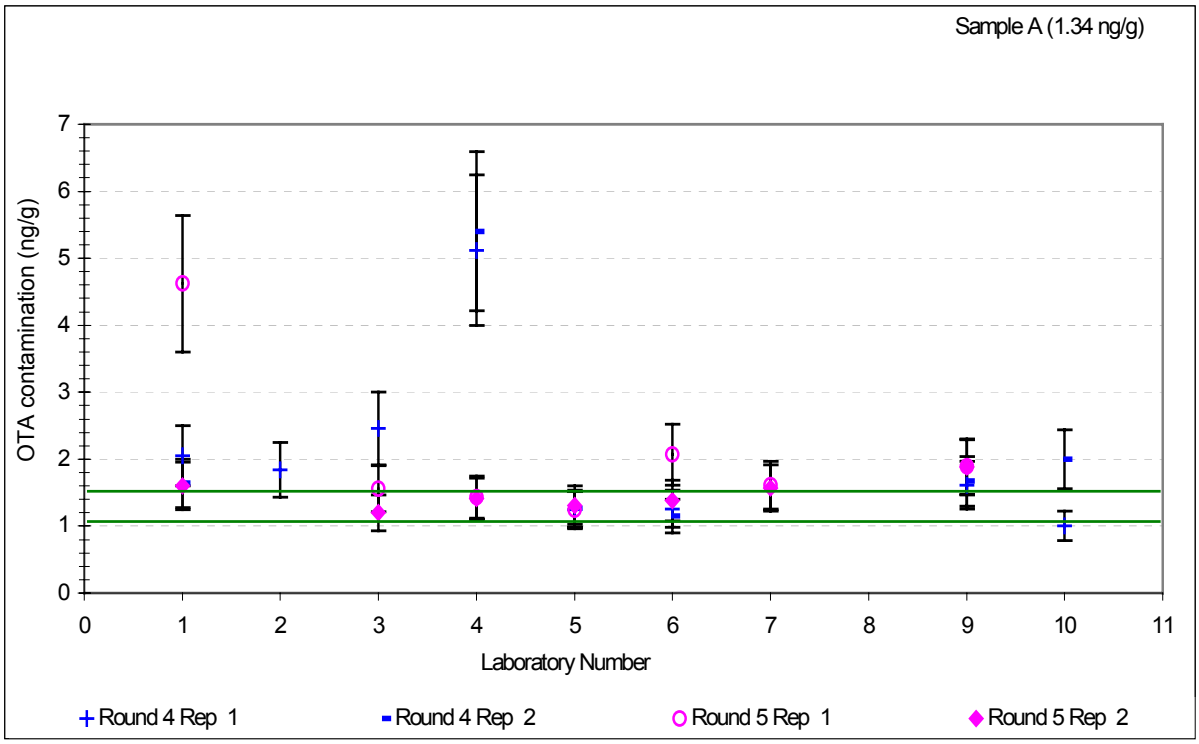
**Note 1:** Results are shown as reported by the laboratories

**Table 8 –** The ochratoxin A results[1] ( ng/g) for the arabica green coffee reference material – Spiked Sample (6.00 ng/g).

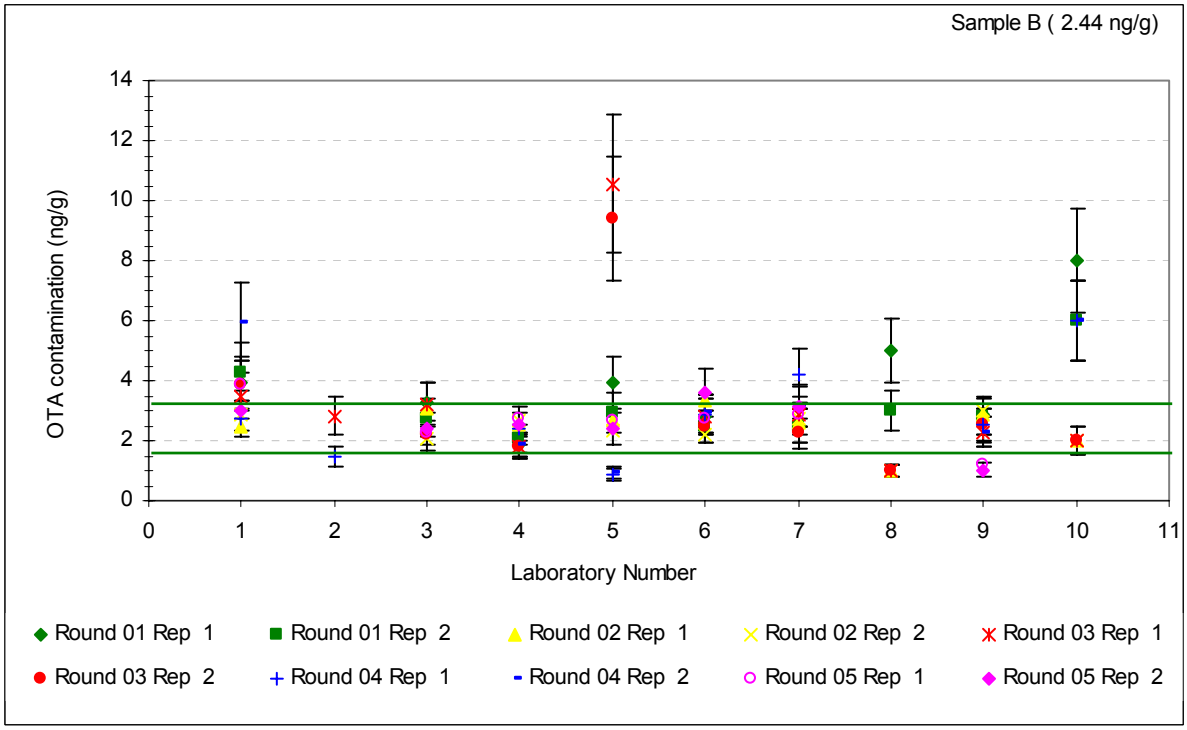
| Laboratory number | First round | | Second round | | Third round | | Fourth round | | Fifth round | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 |
| **1** | 0.34 | 0.88 | 5.74 | ** | 6.33 | 6.16 | 4.55 | 4.64 | 5.49 | 5.69 |
| **2** | - | - | - | - | 7.38 | ** | 2.33 | - | - | - |
| **3** | 4.48 | 5.48 | 5.32 | 5.87 | 5.70 | 5.69 | 2.25 | 2.23 | 4.59 | 4.16 |
| **4** | 2.49 | 2.68 | 4.32 | 4.52 | 4.47 | 4.34 | 3.81 | 4.5 | 4.84 | 4.68 |
| **5** | 2.68 | - | 1.741 | 2.991 | 3.20 | 3.11 | 1.320 | 4.160 | 2.08 | 2.11 |
| **6** | 5.55 | 5.93 | 5.75 | 5.77 | 4.79 | 4.77 | 4.32 | 4.25 | 6.74 | 5.10 |
| **7** | 5.57 | ** | 6.34 | 6.49 | 6.27 | 6.97 | 6.24 | 6.42 | 6.16 | 6.07 |
| **8** | 5.0 | 3.0 | 4 | 4 | 5.30 | 5.30 | - | - | - | - |
| **9** | <0,1 | 5.17 | 6.43 | 6.15 | 5.47 | 5.75 | 6.03 | 6.07 | 5.39 | 5.35 |
| **10** | 4 | 4 | 1 | 1 | 8 | 8 | 6 | 7 | - | - |

[1]Data not corrected by recovery; **–** No results were sent by the laboratory; **Single analysis.

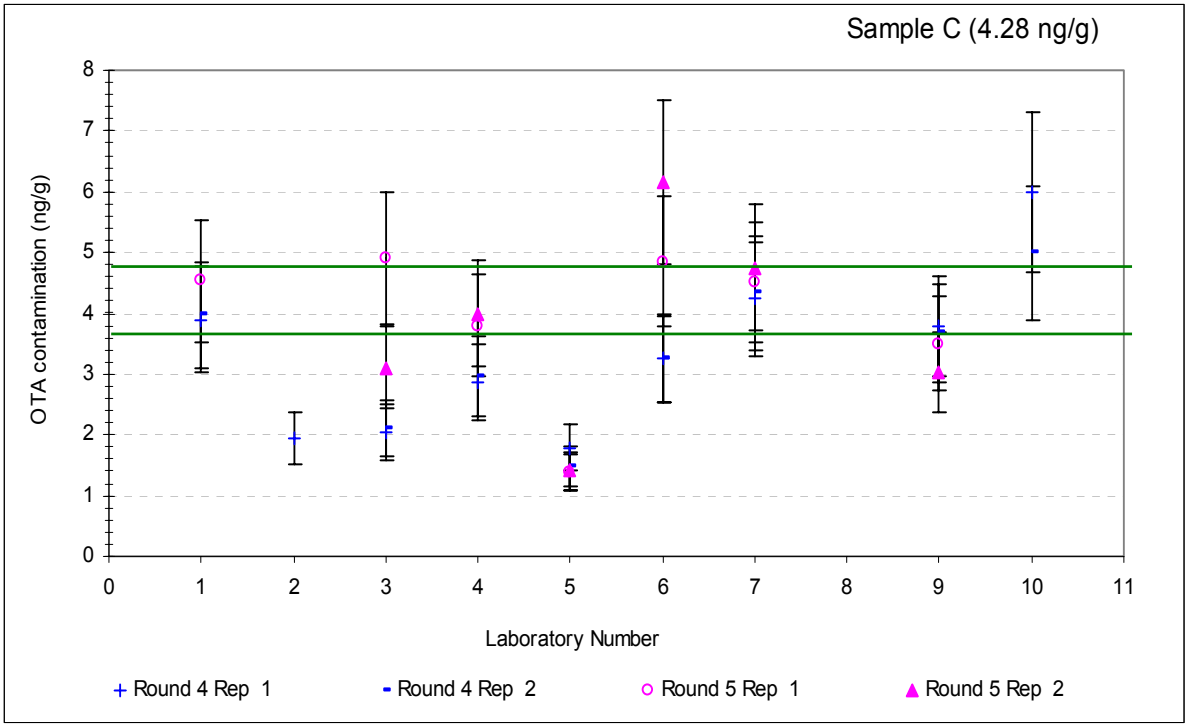
**Note 1:** Results are shown as reported by the laboratories



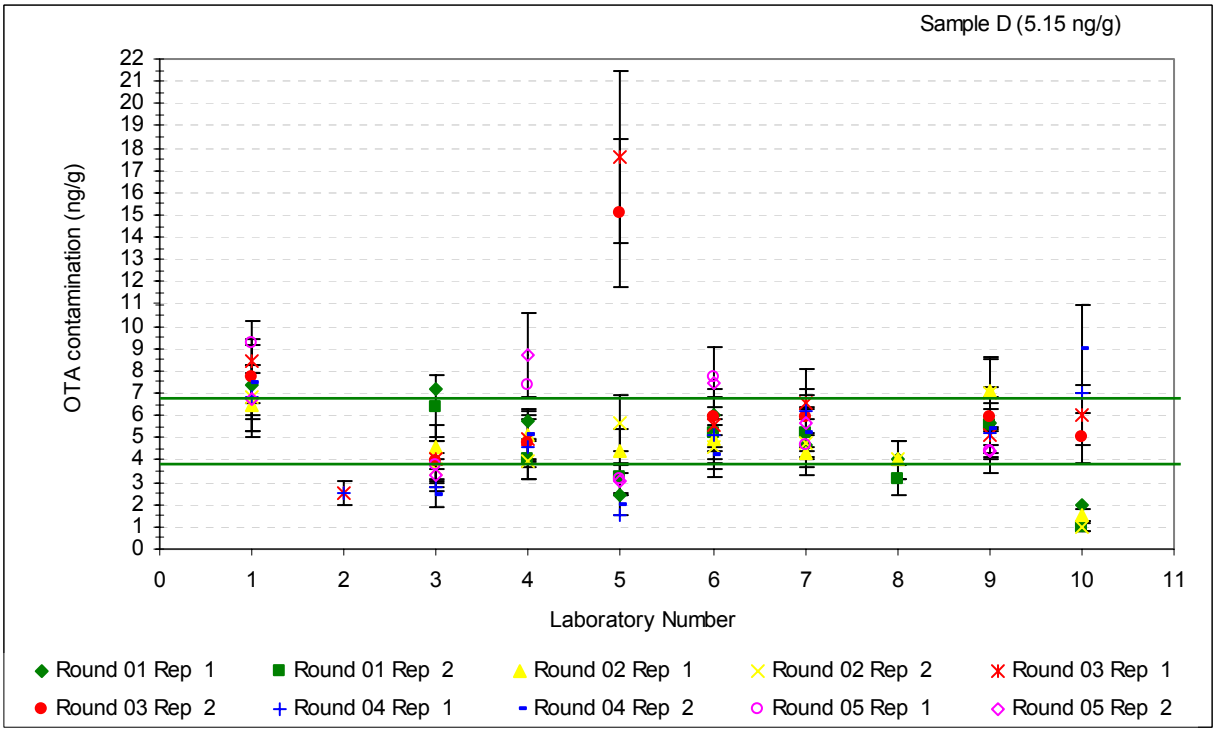

**Graph 1 –** Determination of OTA concentration (ng/g) for **Sample A**, analysed in duplicate, per laboratory, per round: Sample A expected concentration**: 1.34 ± 0.27 ng/g** (1.07 to 1.48 ng/g). Variation between replicates = 22%



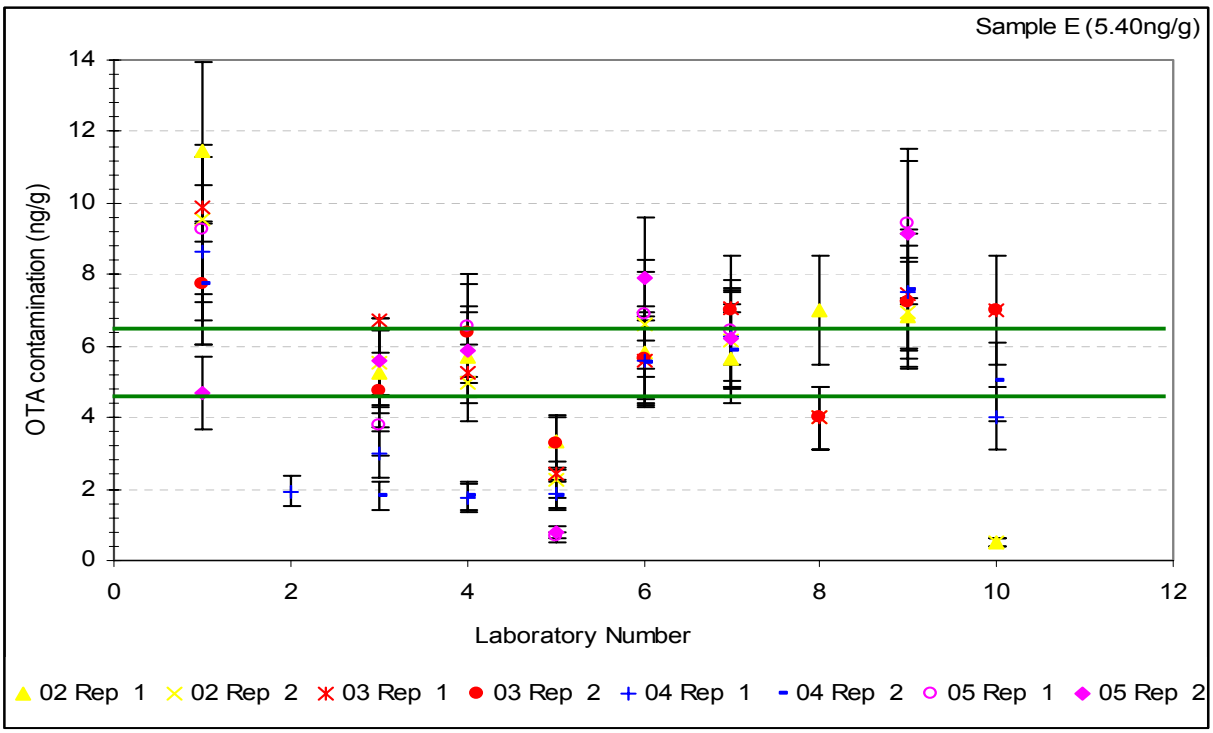

**Graph 2 -** Determination of OTA concentration (ng/g) for **Sample B**, analysed in duplicate, per laboratory, per round: Sample B expected concentration**: 2.44 ± 0.86** ng/g (1.58 to 3.30 ng/g). Variation between replicates = 22%



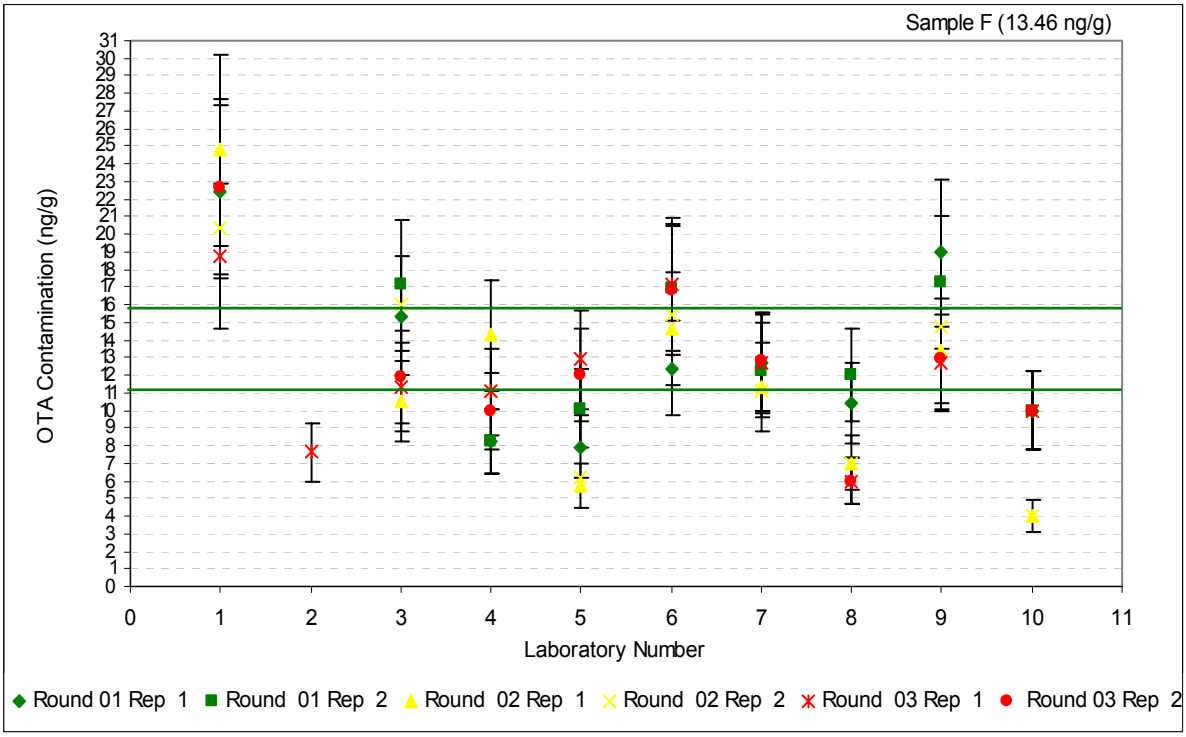

**Graph 3 -** Determination of OTA concentration ( ng/g) for **Sample C**, analysed in duplicate, per laboratory, per round: Sample F expected concentration**: 4.28 ± 0.56 ng/g** (3.72 to 4.84 ng/g). Variation between replicates = 22%



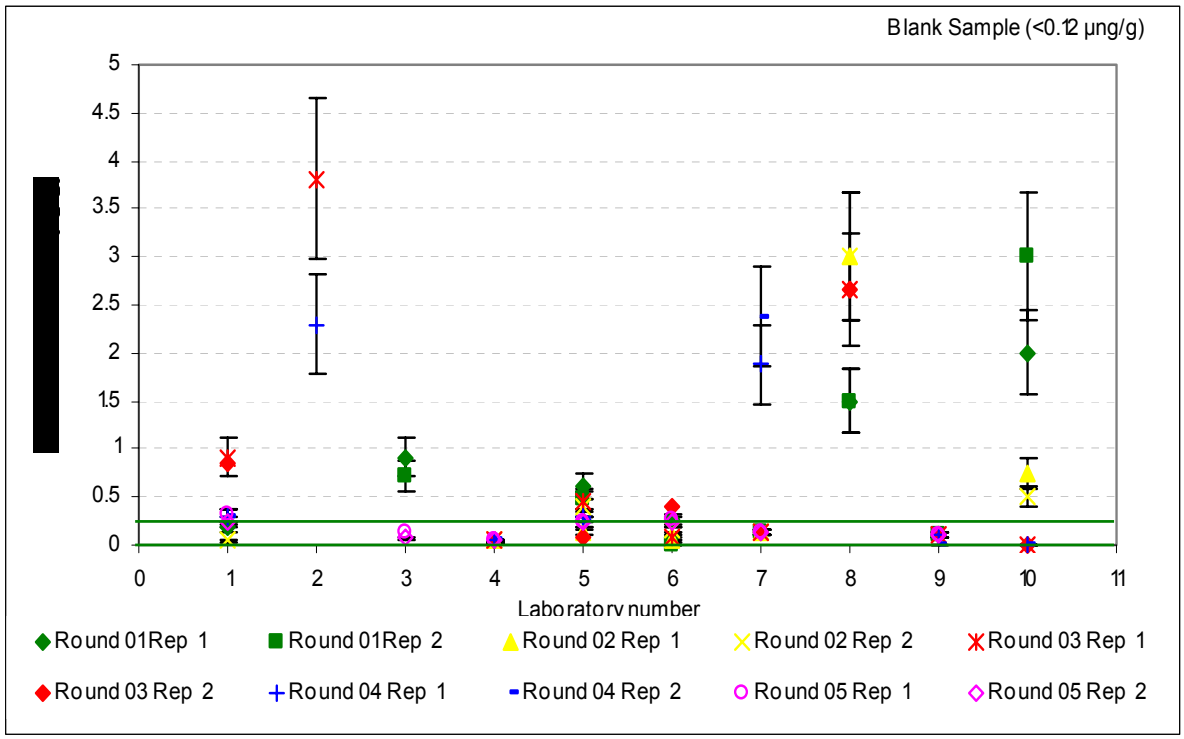

**Graph 4 –** Determination of OTA concentration ( ng/g) for **Sample D**, analysed in duplicate, per laboratory, per round: Sample D expected concentration**: 5.15 ± 1.54 ng/g** (3.61 to 6.69 ng/g). Variation between replicates = 22%



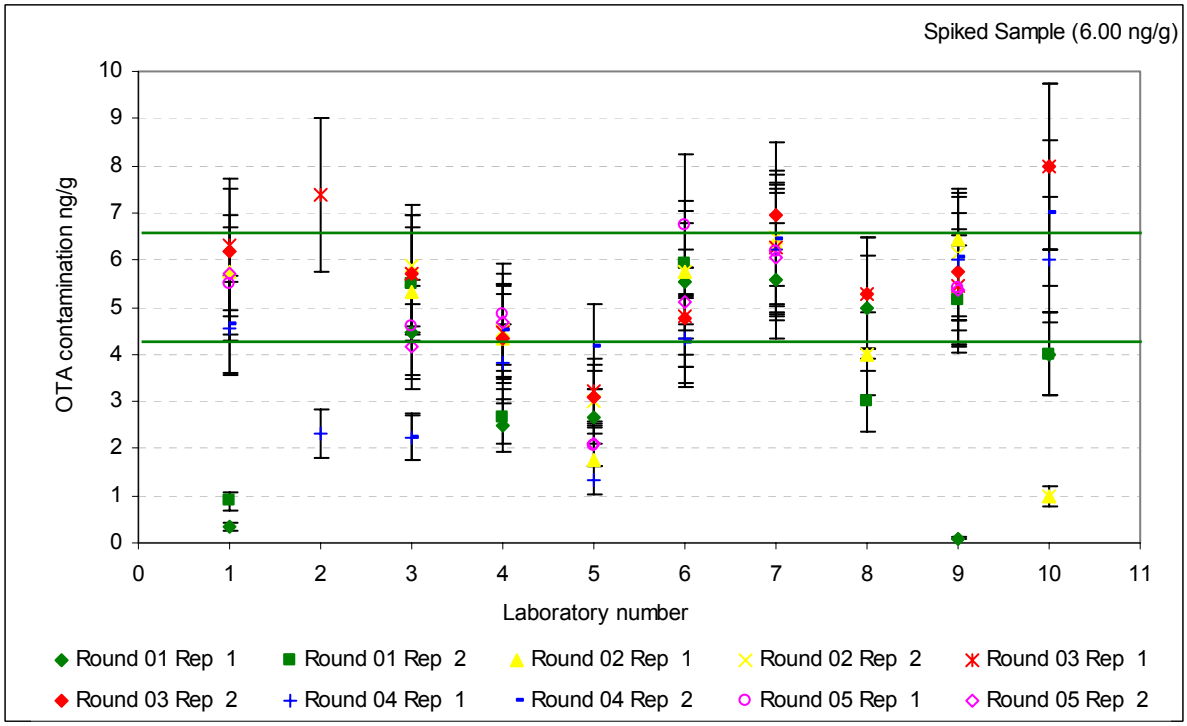

**Graph 5 -** Determination of OTA concentration ( ng/g) for **Sample E**, analysis in duplicate, per laboratory, per round: Sample E expected concentration**: 5.40 ± 1.04 ng/g** (4.36 to 6.44 ng/g). Variation between replicates = 22%



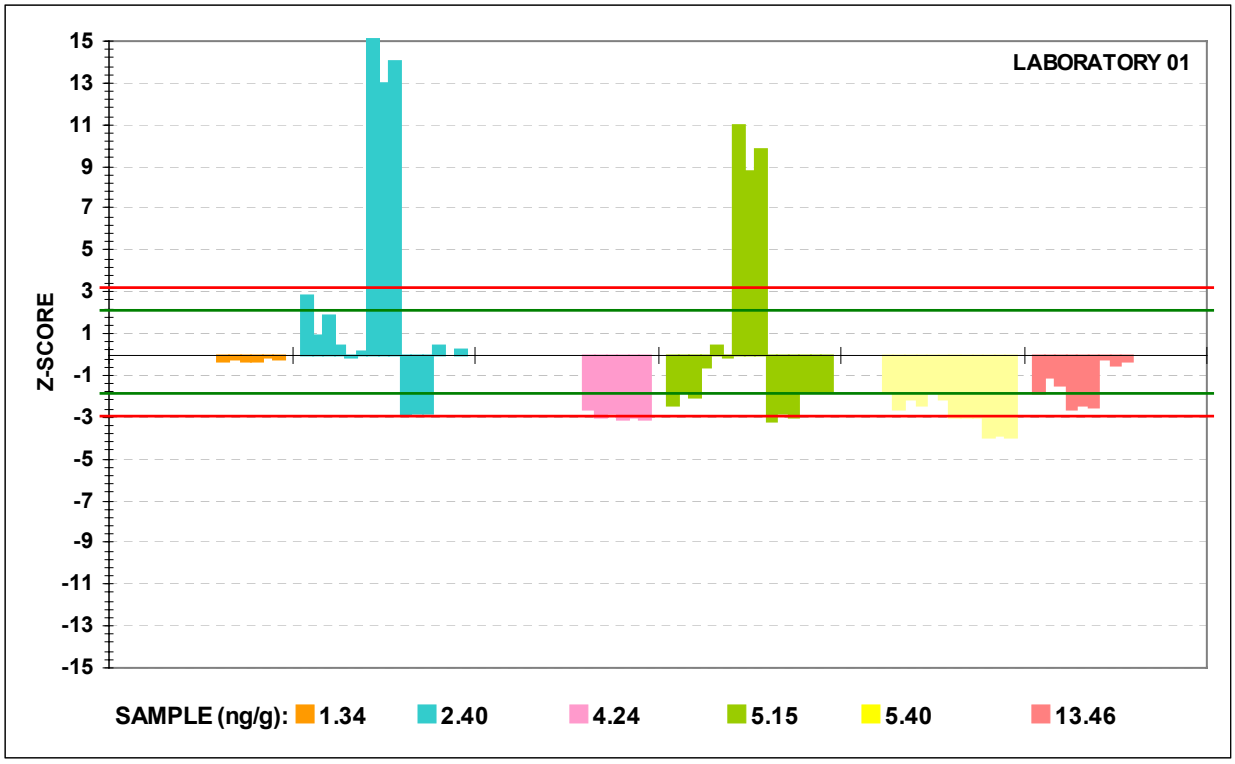

**Graph 6 -** Determination of OTA concentration ( ng/g) for **Sample F**, analysis in duplicate, per laboratory, per round: Sample D expected concentration**: 13.46 ± 2.36 ng/g** (11.10 to 15.82 ng/g). Variation between replicates = 22%



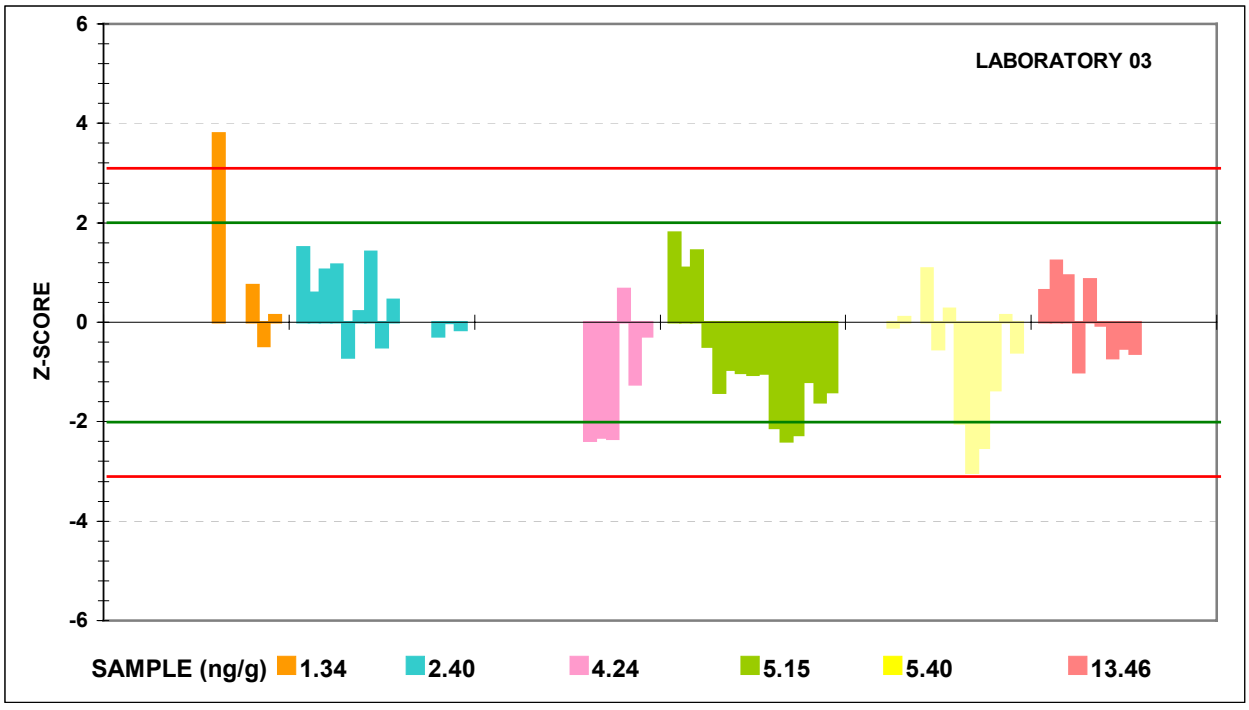

**Graph 7 -** Determination of OTA concentration (ng/g) for Blank **Sample (<0.12 ng/g)**, analysis in duplicate, per laboratory, per round: Variation between replicates = 22%



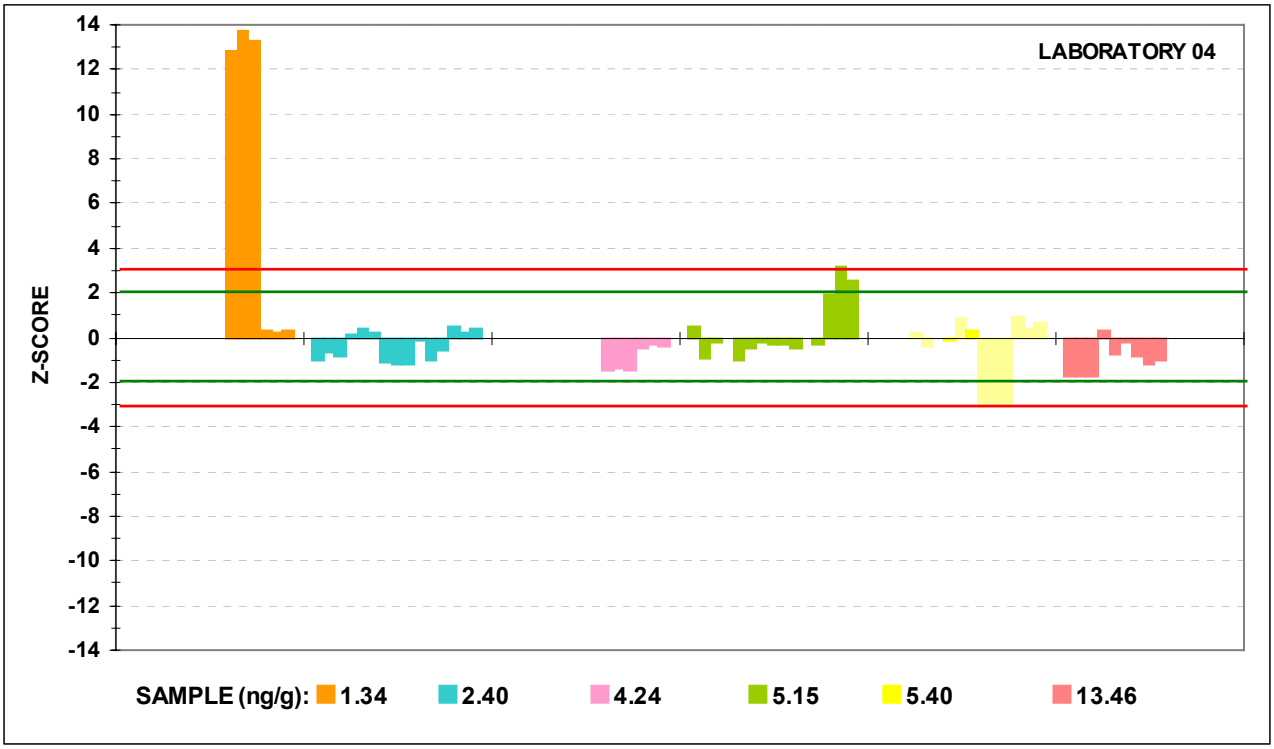

**Graph 8 -** Determination of OTA concentration (ng/g) for Spiked Sample, analysis in duplicate, per laboratory, per round: Spiked sample expected concentration**: 6.00 ng/g. Acceptable range** 4.20 to 6.60.ng/g.



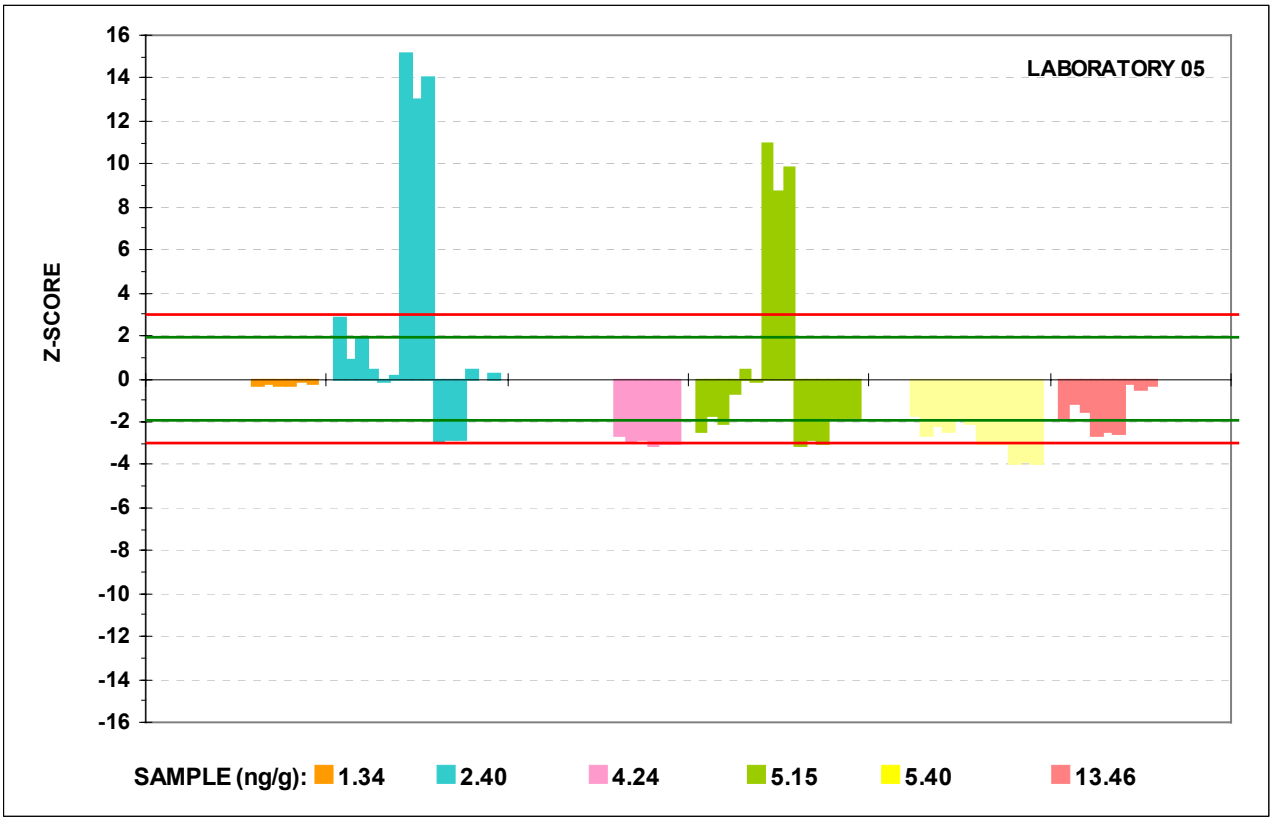

**Graph 9 -** Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 01 for different reference material in five rounds.



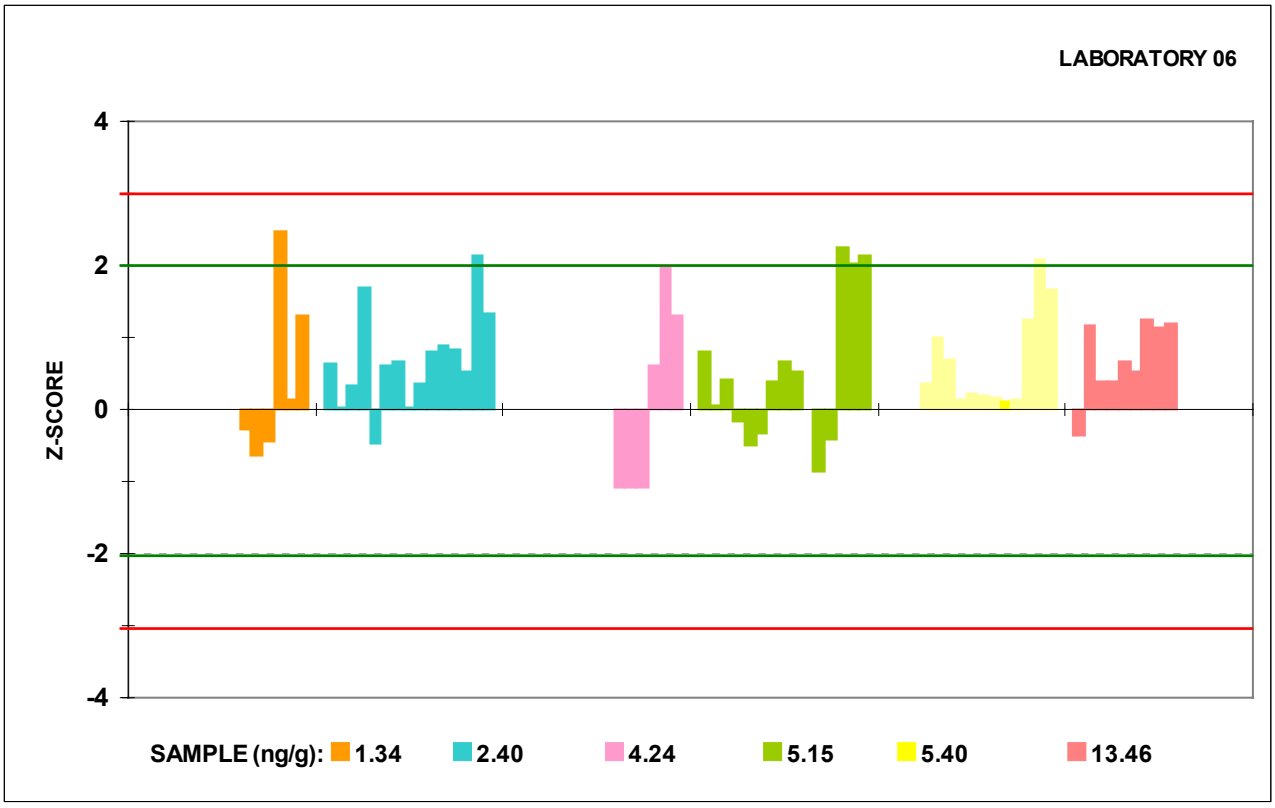

**Graph 10 -** Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 03 for different reference materials in five rounds.



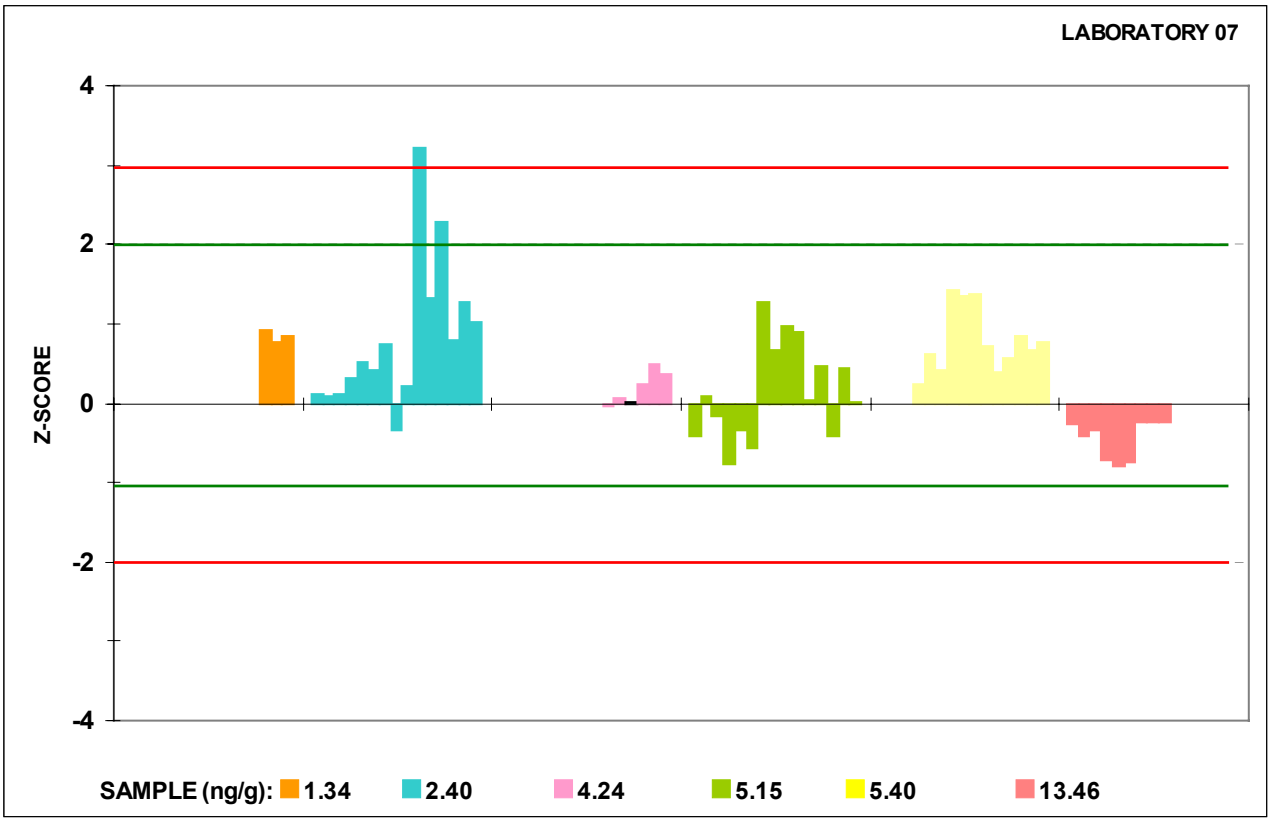

**Graph 11 -** Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 04 for different reference materials in five rounds.



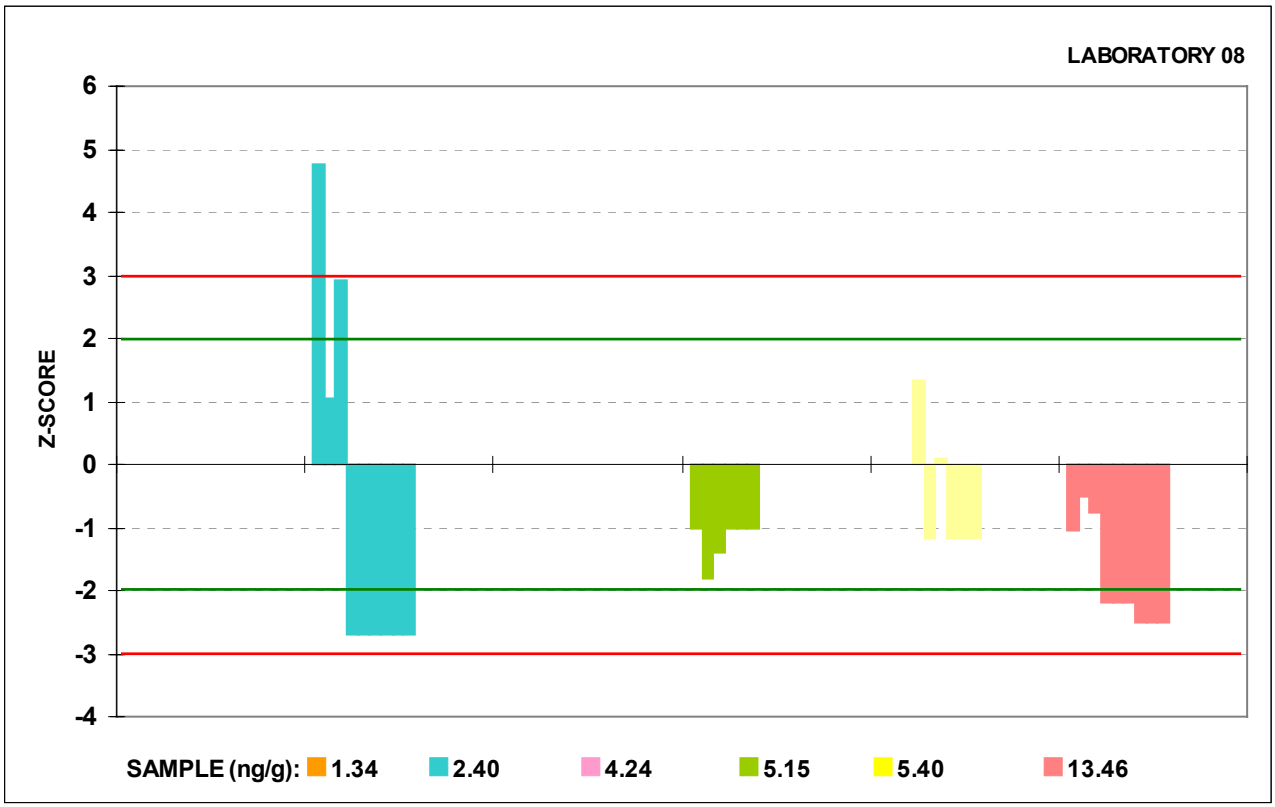

**Graph 12 -** Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 05 for different reference materials in five rounds.



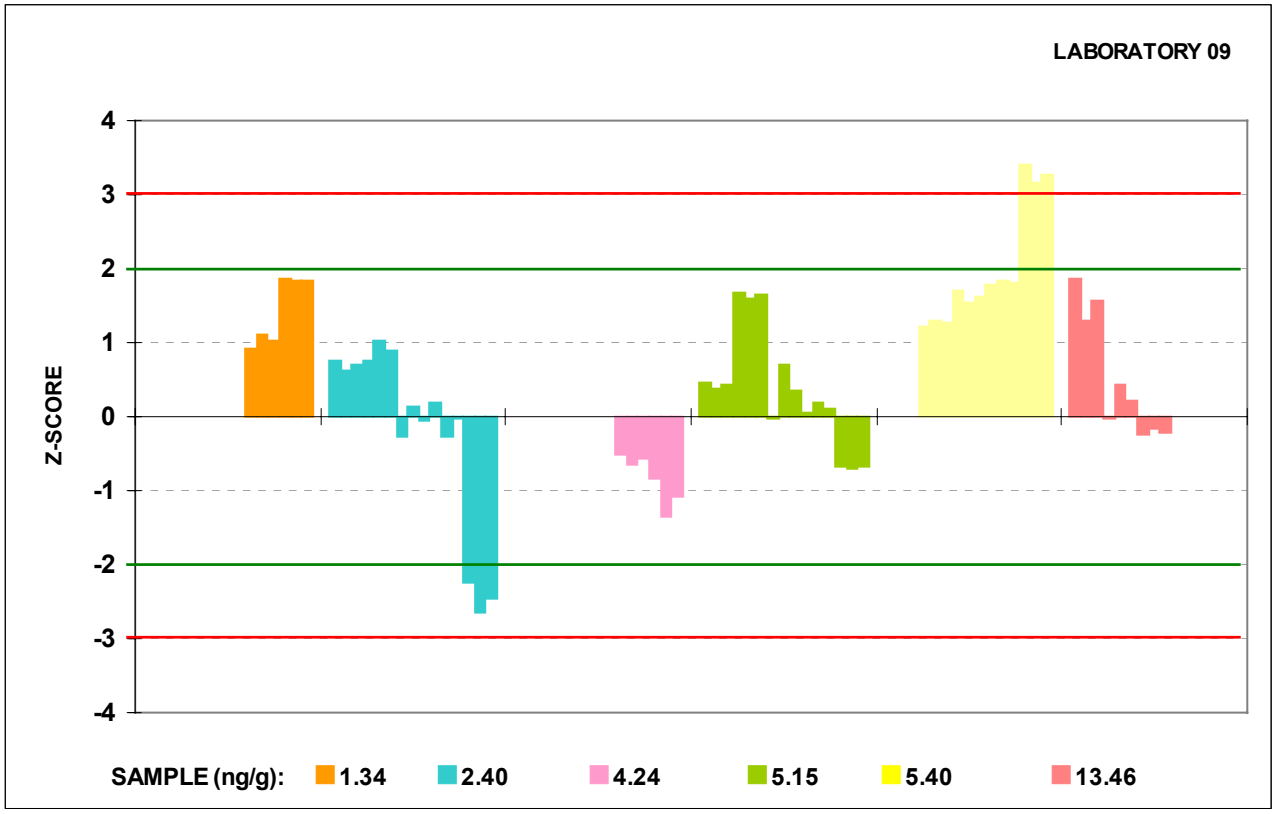

**Graph 13 -** Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 06 for different reference materials in five rounds.



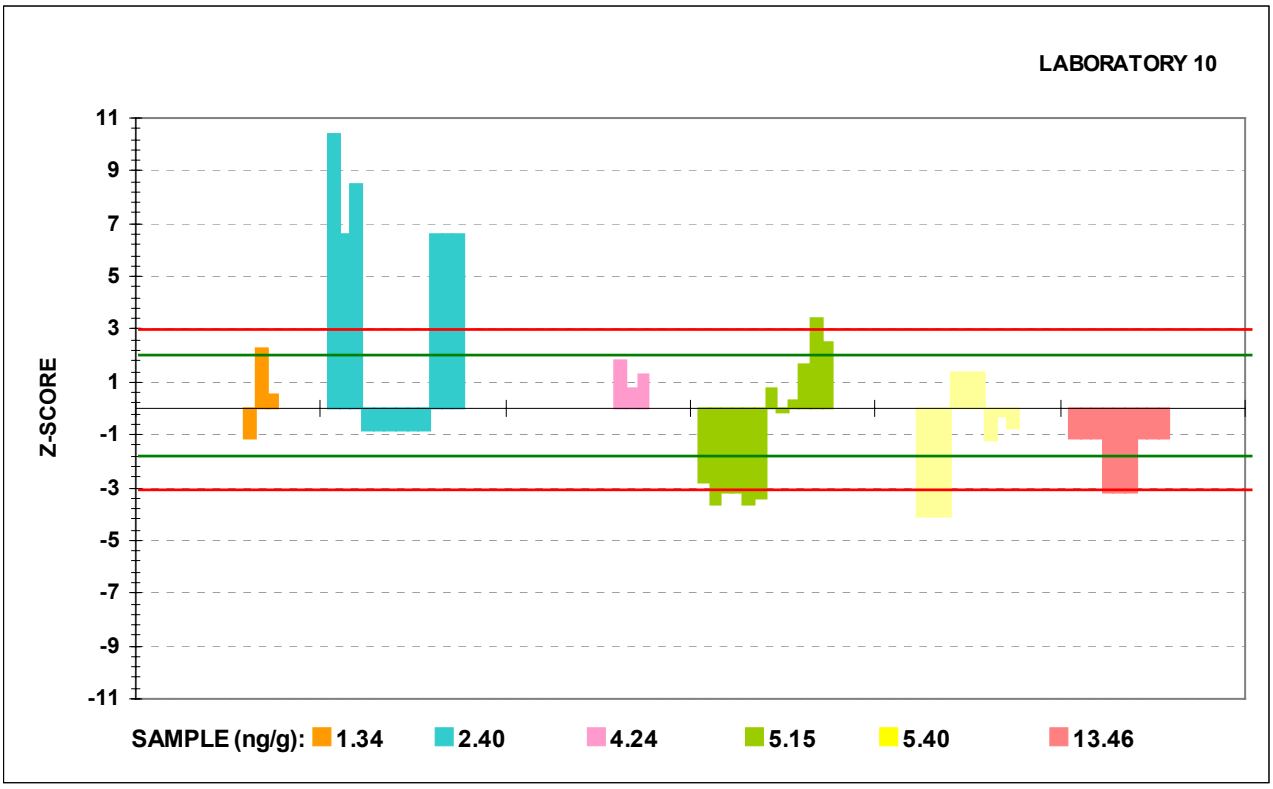

**Graph 14** - Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 07 for different reference materials in five rounds.




**Graph 15** - Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 08 for different reference materials in five rounds.




**Graph 16** - Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 09 for different reference materials in five rounds.




**Graph 17** - Z-score for the ochratoxin A concentration (replicate and mean – ng/g) determinated by LABORATORY 10 for different reference materials in five rounds.

## 6 Discussion

According to Tables 2 to 8 and Graphs 9 to 17 it is possible to observe that:

- **Laboratory 01:** From 63 results reported there were 29 satisfactory results (46%), 20 questionable (32%) and 14 (22%) unsatisfactory results.
- **Laboratory 03:** From 58 results reported there were 48 satisfactory results (83%), 8 questionable (14%) and 2 (3%) unsatisfactory results.
- L**aboratory 04:** From 63 results reported the satisfactory results were 55 (87%), 1 (2%) questionable and 7 (11%) unsatisfactory results.
- **Laboratory 05:** From 63 results reported there were 29 (46%) satisfactory results, 20 (32%) questionable and 14 (22%) unsatisfactory results.
- **Laboratory 06:** From 63 results reported the satisfactory results were 57 (90%) and 06 (10%) are questionable. The Laboratory 06 did not present any unsatisfactory results.

- **Laboratory 07:** From 60 results reported there were 58 (97%) satisfactory results, 01 (1.5%) questionable and 01 (1.5%) unsatisfactory results.

- **Laboratory 08:** From 30 results reported there were 16 (54%) satisfactory results, 13 (43%) questionable and 01 (3%) unsatisfactory results.

- **Laboratory 09:** From 63 results reported there were 57 (90%) satisfactory results, 03 (5%) questionable and 03 (5%) unsatisfactory results.

- **Laboratory 10:** From 48 results reported there were 27 (56%) satisfactory results, 03 (6%) questionable and 18 (38%) unsatisfactory results.

The Graphs 1 to 8 shows the laboratories results, for each reference material taking in consideration the reference material assigned value ($\overline{X}$ ± 2s). A variability of 22%, derived from Horwitz equation [9] was taken as a target value for the duplicates. The evaluation of these dates can be observed in the Table 9.

**Table 9:** Evaluation of results considering the variability ($\overline{X}$ ± 2s) and the repeatability (22%) according to Graphs 01 to 08:

| Laboratory number | Number of reported results | Number of results out of the acceptable range considering the variability of 22% | Number and percents (%) of results out of the acceptable range considering the reference material assigned value ($\overline{X}$ ± 2s) |
|---|---|---|---|
| 1 | 77 | 04 | 56 (73%) |
| 2 | 13 | ** | 12 (92%) |
| 3 | 79 | 0 | 27 (34%) |
| 4 | 83 | 0 | 23 (28%) |
| 5 | 82 | 0 | 63 (77%) |
| 6 | 81 | 0 | 27 (33%) |
| 7 | 77 | 0 | 8 (10%) |
| 8 | 45 | 1 | 30 (67%) |
| 9 | 83 | 1 | 33 (40%) |
| 10 | 64 | 1 | 48 (75%) |

**Single analysis.

## 7 Consideration

There were laboratory delays in returning the *test Material receipt form* as well as *analytical results* to LACQSA during the five proficiency rounds.

It was noticed that some Laboratories have obtained good results for some proficiency rounds and or specifics samples, improving their results in terms of repeatability (22%) i.e. variation between replicates, indicating that there were some standardisation of the analytical procedure. However, in terms of accuracy, some Laboratories have presented results out of acceptable range for both naturally contaminated and spiked samples.

It could be observed, during the five proficiency rounds, an improvement of the Laboratories in following the good laboratory practices concerning the raw data (analytical) record and report.

In some cases there was an indication of inefficiency of the extraction procedure and or losses during clean-up step, probably due to immunoaffinity column flow rate. Some Laboratories have reported problems during the filtration step.

It was observed problems related to standard manipulation during the OTA calibration curve preparation and storage.

Problems related to instrumental use or difficulties in the HPLC quantification step such as setting the chromatographic conditions, calibration curve and or OTA peak integration, and also, injection volume and column pressure can be a possible problem.

Finally the use of TLC together with HPLC is an important tool, once it is an easer and cheaper technique.

## 8 Conclusion

It is important to emphasize that the organization and implementation of the proficiency testing program have allowed to build up a net of laboratories for OTA analysis in the coffee producing countries, including the training of technicians, from different coffee institutions, in analyses of OTA in coffee using both HPLC and TLC quantification. Technical difficulties in some laboratories concerning equipments and skilled technicians exist yet, and need to be solved but the initial laboratory facilities are in place.

Another important contribution of the project was the production of OTA green coffee naturally contaminated reference material that constituted a toll for the adequacy of methods and analytical procedures among coffee producers countries.

## 9 References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[3] AOAC – Association of Official Analytical Chemist. 1998. Natural Toxins. *Official Methods of AOAC International*. Chapter 49. pp. 39-40. 16th edition. 4th revision. (Software Adobe and E-DOC/CJS).

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[6] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. ISO/IEC GUIDE 43-1: Proficiency testing by interlaboratory comparisons – Part 1:Development and operation of proficiency testing schemes. Switzerland. 1997.

[7] IUPAC – International Union of Pure and Applied Chemistry. 1995. IUPAC Harmonized Protocol. *Pure and Applied Chemistry*. **67**. 331-343.

[8] IUPAC – International Union of Pure and Applied Chemistry. 1993. International Protocol for Proficiency Testing of (Chemical) Analytical Laboratories. *Pure and Applied Chemistry.* **60**. 855-864.

[9] THOMPSON. M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relations to fitness for purpose criteria in proficiency testing. *Analyst*. **125**. 385-386.

[10] CEN – European Committee for Standardization. 1999. CEN Report: Food Analysis. Biotoxins: Criteria of analytical methods of mycotoxins. Brussels. *CR 13505:*1999 E. 8p.