



CODA –CERVA

**Belgian National Reference Laboratory
for Mycotoxins in Food and Feed**

Report on the 2009 Proficiency Test for the Determination of Ochratoxine-A in wheat

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Rev. 1 (Nov. 2010) : update of legal references
Rev. 2 (Nov. 2010) : corrections of address LABUG and fig. 1b (error bar Lab01)

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Summary

A Proficiency Test (PT) on the determination of ochratoxine-A (OTA) in wheat was conducted with 11 laboratories in Belgium (8) and abroad (3). One of the laboratories provided two separate sets of results. The sample was a certified reference material (naturally contaminated wheat, ref 31/101) purchased from Bipea..

The assigned value ($X_{\text{ref}} = 35.2 \mu\text{g/kg}$) and the standard uncertainty on this value ($u_{\text{ref}} = 2.9 \mu\text{g/kg}$) were provided by Bipea. The standard deviation for proficiency assessment ($\sigma_p = 8.8 \mu\text{g/kg} = 25\%$) was taken as half the tolerance chosen by Bipea, and is slightly higher than the standard deviation calculated using the modified Horwitz function (22%).

The z-scores were satisfactory for 10 results ($-2 < z < 2$) (among which the nine results obtained after immuno-affinity clean-up) and questionably low ($-3 < z < -2$) for the other two results.

The zeta-scores were satisfactory for 8 results, but too low ($-6.3 < \text{zeta} < -2.9$) for the four lowest results. At least one of the laboratories had probably overestimated the reproducibility of its measurements, but the main reason for the underestimations of uncertainty of measurement seems to be that (negative) biases had been underestimated (overestimation of recovery).

Introduction

Ochratoxin A is a naturally occurring mycotoxin produced by several fungal species in the genus *Penicillium* and *Aspergillus*. It infects a number of feeds and foods (e.g. cereals, grains, coffee, cocoa, dried fruits...) all over the world. The mycelium develops on the fields but produces the mycotoxin during storage.

OTA produced by *Penicillium* and *Aspergillus* fungi are regulated within the European union :

Maximum levels in foodstuffs:

wheat, durum wheat, oats, barley, vine fruits, coffee, baby food... :

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

[Official Journal of the European Union, L 364/5-24, 20.12.2006](#)

Liquorice and spices :

COMMISSION REGULATION (EU) No 105/2010 of 5 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin

[Official Journal of the European Union, L 35/7-8, 6.2.2010](#)

Methods of sampling and analysis

Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

[Official Journal of the European Union, L 70/12-34, 9.3.2006.](#)

Maximum levels in animal feed

COMMISSION RECOMMENDATION (2006/576/EC) of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisines in products intended for animal feeding

[Official Journal of the European Union, L 229/7-9, 23.8.2006](#)

Tolerable daily intake

- (http://www.internationalpbi.it/docs/Micotossine_OCRATOSSINAA.pdf)

Joint FAO/WHO Food Standards Programme, CODEX Committee on food additives and contaminants, *Thirty-first Session, The Hague, The Netherlands, 22-26 March 1999*, Position paper on ochratoxin A

- (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620762138.htm)

Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A in food, *The EFSA journal* 365, 1-56 (2006)

Test material and instructions to participants

There were eleven participants, including the NRL. Nine were laboratories approved by the Belgian Federal Agency for the Safety of the Food Chain (FASFC). The remaining two laboratories participated on a voluntary basis.

The test material was a certified reference material (naturally contaminated wheat, ref 31/101) purchased from Bipea. The original bag, containing each approximately 700 g of wheat flour test material, was opened, remixed and distributed in bags of approximately 55gr. Those were labelled and kept frozen until they were dispatched to the participants on June 22, 2009. Each participant received one bag.

The participants were invited to follow their routine procedures for the determination of ochratoxine and report (see annexes) :

- the values of two independent measurements, corrected for recovery
 - the recovery factor used for correction
 - an estimate of their uncertainty of measurement (u) and the coverage factor (k) used
 - short descriptions of the purification and measurement methods
- The results were to be reported on a form provided in attachment, in the same manner (eg. number of significant figures) as when reporting to customers.

Reference values

The reference value X_{ref} , and its standard uncertainty u_{ref} were respectively 35.2 µg/kg and 2.9 µg/kg, as reported by Bipea. They are the robust mean and the robust standard deviation of the results of 9 out of the 33 laboratories having reported a result for ochratoxine in their PT nr 31/101. The standard deviation for proficiency assessment ($\sigma_p = 8.8 \mu\text{g/kg} = 25\%$) was taken as half the tolerance chosen by Bipea for their PT (17.6 µg/kg). This is between the standard deviations calculated from the Horwitz equation (26.5%) and the Horwitz-Thompson equation (22%). (M. Thompson (2000) Analyst, 125, 385-386).

Results and discussion

Most participants reported their results before deadline (Aug. 28), but the last report from the participants on a voluntary basis was received on Oct. 23.

All participants reported two measurement values, and all except one reported their recovery. All provided an estimate of their measurement uncertainty, and all except three reported that the coverage factor for this estimate was k=2 (for the calculation of the zeta-scores it was assumed that the coverage factor used by the remaining three laboratories was also k=2).

Eight laboratories used immuno-affinity clean-up (IAC). Six of these used fluorimetric detection (LC-FL), one used MS-MS detection (LC-MS-MS) and one used both LC-FL and LC-MS-MS and reported two separate results. Of the remaining three laboratories, two used LC-MS-MS without IAC clean-up, and one applied the ISO 15141-2 (1998) method (bicarbonate clean-up followed by LC-FL)

Individual laboratory performance is expressed in terms of z and zeta scores in accordance with ISO 13528 and the International Harmonised Protocol.

$$Z = \frac{X_{lab} - X_{ref}}{\sigma_p}$$

$$Zeta = \frac{X_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}}$$

where

X_{lab} is the measurement result reported by a participant

X_{ref} is the assigned value

σ_p is the "standard deviation for proficiency assessment"

u_{ref} is the standard uncertainty on the reference value

u_{lab} is the standard uncertainty reported by a participant

(all in $\mu\text{g}/\text{kg}$ – note that for the calculation of the zeta-scores the values of u_{lab} in $\mu\text{g}/\text{kg}$ were recalculated from the u_{lab} as reported in %, in order to avoid errors due to the rounding of the u_{lab} as reported in $\mu\text{g}/\text{kg}$)

The laboratory codes were attributed by increasing value of z-score. Each lab code was communicated confidentially to the corresponding participant. The results reported by the participants and the z- and zeta-scores are summarized in table 1 and figure 1.

Visual examination of figure 1a ("kernel plots") shows that the distribution of results is distinctly 3-modal :

- 6 participants reported results close to the expected value (z-scores between -0.9 and -0.3)
- 4 participants reported lower results (z-scores between -2.3 and -1.7)
- 2 participant reported higher results (z-score between 1.1 and 1.7)

Most of these results were in the acceptable range ($-2 < z < 2$). Only the two lowest were in the questionable range ($-3 < z < -2$).

It is worth mentioning that in this PT the methods based on IAC clean-up performed better (z-scores between -1.7 and 1.1) than those without IAC clean-up (z-scores - 2.3, -2.2 and 1.7).

Most laboratories (L05 to L12) also obtained good zeta-scores, i.e. their estimates of their measurement uncertainty is such that the 95% confidence interval around their result includes the reference value, or at least part of the 95% confidence interval around it (see figure 1b).

The other laboratories, L01 to L04, underestimated their measurement uncertainty and obtained zeta-scores between -6.3 and -2.9. In the case of L03, the reported MU (2.9%, $k=2$) seems unrealistically small: actually it is even smaller than the RSD between the two results provided (5.9%). In the three other cases the reported MU seem more realistic at first sight (21% to 41%, $k=2$) but are smaller than the deviations from the reference value (-58% to -42%).

For the end-user, zeta-scores lower than -2, such as those obtained by laboratories L01 to L04, mean a risk of wrong decisions due to "false negatives" or "false compliants", i.e. of accepting a lot of food or feed which should have been rejected.

Table 1 : Values as reported by the participants and scorings calculated by the organiser

lab code	data as reported by the participants :						calculations :		
	result 1 (µg/kg)	result 2 (µg/kg)	recovery (%)	uncertainty (µg/kg)	uncertainty (%)	k (coverage factor)	average (µg/kg)	z-scores	zeta-scores
L01	15.82	13.48	70% - 68%		3.06%		14.65	-2.34	-7.07
L02	16	16		5	30%		16.00	-2.18	-5.10
L03	19.4	21.1	92.9% - 86.1%		2.9%	2	20.25	-1.70	-5.13
L04	20.3	20.5	98%	8.4	41%	2	20.40	-1.68	-2.91
L05	27.17	27.03	94.64%		25%	2	27.10	-0.92	-1.82
L06	29.9	27.8	81.7%		40%	2	28.85	-0.72	-0.98
L07	30.8	31.1	101.5% - 102.5%	3.5	11.4%	2	30.95	-0.48	-1.25
L08	31	31	98.2%	4	12.5%		31.00	-0.48	-1.20
L09	27.12	35.15	98%		34.1%	2	31.14	-0.46	-0.67
L10	32.69	33	86.6%		40%	2	32.85	-0.27	-0.33
L11	45.04	44.10	107.9%	10.70	24%	2	44.57	1.06	1.54
L12	47.9	52.1	95.4%	13.2 - 14.3	27.5%	2	50.00	1.68	1.98

Figure 1a :
Ochratoxine-A (OTA)
in wheat flour ($\mu\text{g}/\text{kg}$),
kernel density plot

(vertical lines :
solid : X_{ref}
dashed : $X_{\text{ref}} \pm 2 \cdot u_{\text{ref}}$
dotted : $X_{\text{ref}} \pm 2 \cdot \sigma_p$

with
 $X_{\text{ref}} = 35.2 \mu\text{g}/\text{kg}$;
 $u_{\text{ref}} = 2.9 \mu\text{g}/\text{kg}$
 $\sigma_p = 8.8 \mu\text{g}/\text{kg}$)

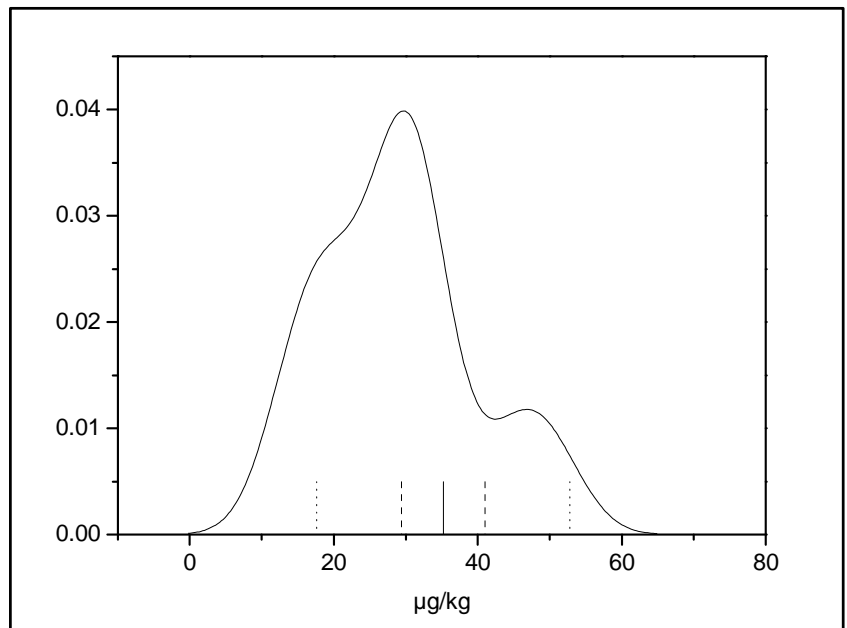


Figure 1b :
Results and
95% confidence intervals
(expanded uncertainty),
as reported by participants

(horizontal lines :
solid : X_{ref}
dashed : $X_{\text{ref}} \pm 2 \cdot u_{\text{ref}}$
dotted : $X_{\text{ref}} \pm 2 \cdot \sigma_p$)

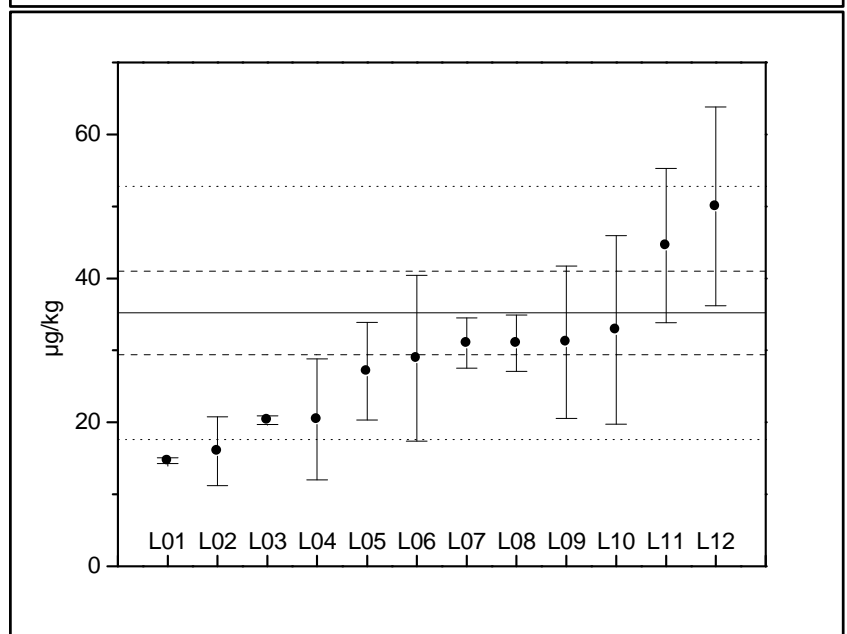
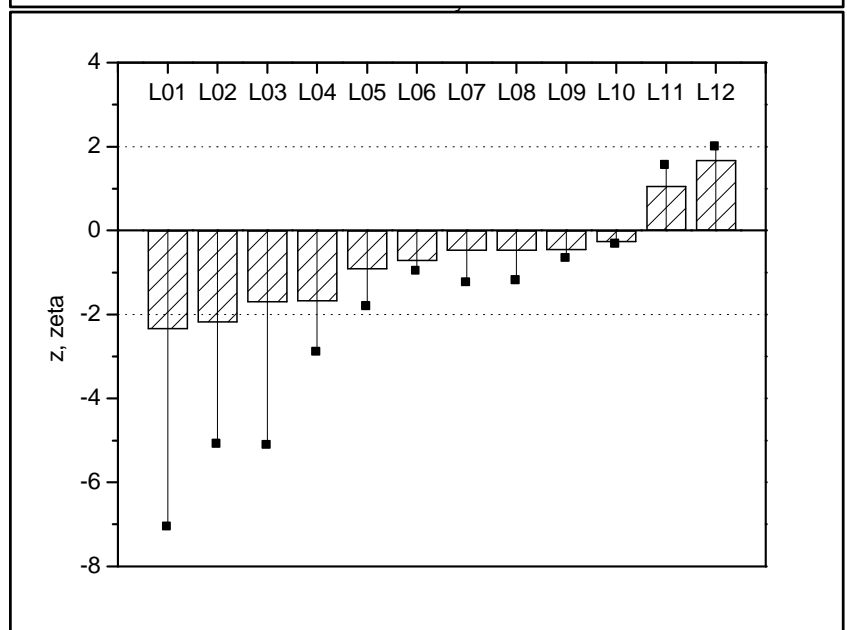


Figure 1c : z- and zeta-scores

$$z = (X_{\text{lab}} - X_{\text{ref}}) / \sigma_p$$

$$\text{zeta} = (X_{\text{lab}} - X_{\text{ref}}) / \sqrt{u_{\text{ref}}^2 + u_{\text{lab}}^2}$$



Annexes

List and addresses of the participating laboratories (by alphabetical order) :

Organisation	Address	
CER Marloie	Rue du Point du Jour, 8	B-6900 Marloie
CERVA-CODA	Leuvensesteenweg, 17	B-3080 Tervuren
FLVVT	Leuvensesteenweg, 17	B-3080 Tervuren
Fytolab	Technologiepark, 2/3	B-9052 Zwijnaarde
Handelslaboratorium b.v.	Coolhaven, 32, postbus 6003	NL-3002-AA Rotterdam
INRAP	Technopole Sidi Thabet,	TN-202 Ariana (Tunisie)
LABUG, Lab voor Bromatologie	Harelbekestraat, 72	B-9000 Gent
ISP-WIV	Rue Juliette Wijsmans,	B- Bruxelles
NRL-RO Brno, UKZUZ	Hroznova 2	CZ-65606 Brno (Czech Rep.)
OLEOTEST	Lage weg, 427	B-2660 Antwerpen
SGS-Belgium	Polderdijkweg, 16 Haven 407	B-2030 Antwerpen

Letter to the participants :



V A R

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

Laboratoire HPLC-INRAP, *Unite Mycotoxine*
A l'attention de *Mrs Amira ANENE*
Technopole Sidi Thabet
TN - 2020 Ariana

Tunisie

Your reference

Our reference
181/131/JCM/WD

Your contact: Jean-Claude Motte
tél.: +322.769.22.80
jemot@var.fgov.be

Date
august, 18th 2009

SUBJECT: Ring Test OTA in Wheat

ANNEXES: 4

Dear Participant,

In the annexes you will find:

- annexe 1 : description of the sample and instructions about the results to be sent back.
- annexe 2 : the test-sample (grinded feed grains)
- annexe 3 : Receipt form of the sample
- annexe 4 : results form

Annexe 3 is a receipt form. Please fill the form and return it us by FAX (FAX n°: 02/769.23.05) as soon as you receive the sample.

Annexe 4 is for the results of the analysis. Please enter here the name of the analyst, the name of your laboratory, the sample code, for each replicate the calculated concentration for OTA corrected for recovery and the recovery. **Do not forget to fill in the uncertainty (u).**

Also give a short description of the used method as for test-report for both purification step and measurement (LC...).

After checking all the data you have entered, first submit the form electronically to the proposed Email. Afterwards you may save the form on your PC, print it, sign the hardcopy and fax or mail it to the address shown on the form. The deadline for reporting back results is August 28th, 2009 (we give a longer period of ten weeks taking into account the summer holidays).

As I am the project leader for this inter-laboratory comparison please do not hesitate to contact me in case of questions or doubts.

Your participation is free of charge.

Yours sincerely,

Dr. Sc. Jean-Claude Motte

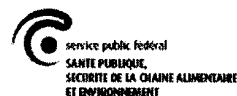
Remember: So far this test is organised in collaboration with the FASFC (AFSCA-FAVV), please note that you have the obligation to participate.

----- Scientific research at the service of safe food production and animal health -----

Central office
Veterinary departements:
Groeselenberg 99, B-1180 Brussels
Tel. +32 (0)2 379 04 00
Fax +32 (0)2 379 06 70 (general)
+32 (0)2 379 04 01 (director)
+32 (0)2 379 06 64 (dispatching)

Agrochemical departements:
Leuvensesteenweg 17
B-3080 Tervuren
Tel. +32 (0)2 769 22 00
Fax +32 (0)2 769 23 05

Experimental centre
Kerklaan 68
B-1830 Machelen
Tel. +32 (0)2 251 33
26
Fax +32 (0)2 251 00
12



be

e-mail info@var.fgov.be - <http://www.var.fgov.be>



Ring-Test "Ochratoxine A"

Annexe 1: description

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the validity of the particular testing method.

The purpose of VAR is to offer proficiency tests for selected parameters in concentrations with practical relevance. Realisation and evaluation of the present proficiency test is based on the principles of the ISO guide 43 (1997).

2. Realisation

2.1. Test material

The test material is grinded feed grains obtained in bulk from an accredited society known for their ring test organisation. The sample is sent in 52gr portion stocked at -18°C. Mailing and manipulation may occur at room temperature.

2.2. Test

One portion of test material will be sent, from CERVA-Tervuren, to every participating laboratory in the last week of June 2009. The testing method should be the laboratory routinely method used for OTA determination. The tests should be finished at 28th of august the latest.

If you are unable to finish for this datum, please send us a mail with the forecast datum.

2.3. Results

The participants are requested to submit 2 independent measurements. The results will be submitted on the standard form (see page 2). The returned results will be: results corrected by recovery for both replicates, recovery, average and uncertainty (u). The results should be reported in the same way (e.g.: number of significant figures) as usually reported for the customers.

Please report the average of the results with technique, short method description and uncertainty (u) information in the allocated space on the results form.

Please do not report uncertainty on each individual measurement.

Keep care: in case you report combined uncertainty (U) do not forget to state your coverage factor.



RING – TEST OCHRATOXINE A VAR 06/2009

Please send to : J.C.Motte , FAX n° +322.769.23.05

Annexe 3: receipt form

Name of the Laboratory :	
Name of the analyst :	

Sample number :	0631 - _____		
Date of reception of the sample :			
State of the received sample :	Good	Bad	Open

Date	stamp	Signature



RING – TEST OCHRATOXINE A VAR 06/2009
 SUBMIT RESULTS TO : jemot@var.fgov.be

Annexe 4: results form

DEADLINE :	28 th of august 2009
Name of the Laboratory :	
Name of the analyst :	

Parameter	Results corrected for recovery	Units	Recovery as %	uncertainty (u)	
VAR- testnumber	0631-			Units	
<i>Ochratoxin A</i>				µg/kg	%
Replicate 1		µg/kg			
Replicate 2		µg/kg			

	Yes	no
Recovery in the same matrix		

Short method description as for test-report :	
Purification :	
Measurement:	

Datum :

Stamp :

Signature :