



CODA –CERVA

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

**Report on the 2008 Proficiency Test for the
Determination of Deoxynivalenol in wheat**

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Summary

A Proficiency Test on the determination of deoxynivalenol (DON) in wheat was conducted with 9 laboratories in Belgium. The sample was a certified reference material (naturally contaminated wheat, ref T2238) purchased from FAPAS (CSL-GB).

The assigned value ($X_{\text{ref}} = 1017$ ppb) and the standard uncertainty on this value ($u_{\text{ref}} = 21.2$ ppb) were provided by FAPAS. The standard deviation for proficiency assessment ($\sigma_p = 16\% = 162$ ppb) was calculated using the modified Horwitz function.

The z-scores were satisfactory for 7 laboratories and borderline satisfactory-questionable ($z \approx -2$) for 2 laboratories. The zeta-scores were satisfactory for 6 laboratories, borderline satisfactory-questionable ($z \approx 2$) for 1, unsatisfactory ($z < -3$) for 2. At least one of these two 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

Deoxynivalenol (DON) is a naturally occurring mycotoxin produced by several fungal species in the genus *Fusarium*. It infects wheat and other small grains such as barley and corn. DON is most often associated with cool and wet environmental conditions where *Fusarium* tends to thrive. Don toxicosis is associated with feed refusal, vomiting and reproductive problems in animals.

DON and other mycotoxins produced by *Fusarium* species are regulated within the European Union (EC 1881/2006, Official Journal L364, 20/12/2006, 0005-0024).

Test material and instructions to participants

There were nine participants, including the NRL. Seven 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 T2238) purchased from FAPAS (CSL-GB). The bags, containing each approximately 55 g of wheat flour test material, were re-labelled but not opened, and kept frozen until they were dispatched to the participants on June 23, 2008. Each participant received one bag.

The participants were invited to follow their routine procedures for the determination of deoxynivalenol 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 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 1017 ppb and 21.2 ppb, as reported by FAPAS. They are the mode and the standard error on the mode, as calculated by FAPAS from the results of 60 of the 80 participants to their PT 2238. The standard deviation for proficiency assessment ($\sigma_p = 16\% = 162$ ppb) was calculated from the modified Horwitz function (M. Thompson (2000) *Analyst*, 125, 385-386).

Results and discussion

All participants reported their results before deadline (Aug. 31). All reported two measurement values and an estimate of their measurement uncertainty. All except one reported their recovery.

Since there was some doubt concerning the coverage factors on which the uncertainties as reported were based, the participants were asked by e-mail to confirm these coverage factors (last answer received Oct. 24).

Four laboratories used immuno-affinity clean-up (IAC) and liquid chromatography with UV detection (LC-UV). One used clean-up on "Mycosep Trich 227" column and LC with post-column derivatisation and fluorimetric detection (LC-FL). Three used LC-MS without column clean-up. One used ELISA.

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

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 -0.46 and 0.14)
- 2 participants reported lower results (z-score around -2)
- 1 participant reported a higher result (z-score around 1.4)

Among the 6 results close to the expected value, 2 were obtained with IAC-LC-UV, 2 with LC-MS, 1 with LC-FL and 1 with ELISA. Due to the limited number of participants, no attempt was made at a detailed interpretation of the results as a function of the methods used.

Figure 1b shows clearly the danger in underestimating the measurement uncertainty : although the results of participants L01 and L02 are not unsatisfactory (z-score around -2), the 95% confidence intervals calculated from their estimates of their measurement uncertainty does not include the "real" value V_{ref} . This translates into zeta-scores (-6.2 and -3.5 respectively) far away from the acceptable range (figure 1c).

Although two replicate measurements on one sample are of course quite insufficient as data set for estimating the uncertainty, it may be useful to at least compare the RSDs and average biases of these two replicates to the laboratories' own estimates of their measurement uncertainty :

- the RSD of L02 was 17.9%, much higher than their estimate of their standard uncertainty (12.5%)
- the average biases of L01 and L02 were -33.4% and -31.3%, again much higher than their estimates of their standard uncertainties (7.5% and 12.5%)

This suggests that L02 overestimated its reproducibility and that both L01 and L02 underestimated their (negative) biases (possibly by overestimation of their recoveries and/or underestimation of matrix effect on the detection).

Conclusions

All participants provided satisfactory or nearly satisfactory results (z-scores between -2.1 and + 1.4). However, two participants apparently underestimated their uncertainty of measurement, in a manner that could lead the end-user of the results to wrong decisions ("false negatives" or "false compliants", i.e. accepting a lot of food or feed which should have been rejected).

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

lab code	result 1 (ppb)	result 2 (ppb)	average (ppb)	recovery (%)	recovery in other matrix (%)	uncertainty (ppb)	uncertainty (%)	k (coverage factor)	Z-scores	zeta-scores
L01	689	665	677	89.0%		102	15.0%	2	-2.09	-6.16
L02	788	611	699	110.0%	91.0%	175	25.0%	2	-1.96	-3.53
L03	935	951	943	102.2%		124	13.2%	2	-0.46	-1.13
L04	905	1020	963	87.3%		346	35.9%	2	-0.33	-0.31
L05	975	956	966	103.5%		280	28.0%	2	-0.31	-0.36
L06	955	1000	977.5	98.1%		219	22.4%	2	-0.24	-0.35
L07	998	974	986	92.2%		168	16.5%	2	-0.19	-0.36
L08	990	1100	1040			187	18.0%	2	0.14	0.24
L09	1253	1242	1248	95.0%		112	9.0%	1	1.42	2.03

Figure 1a :
Deoxynivalenol (DON)
in wheat flour (ppb),
kernel density plot

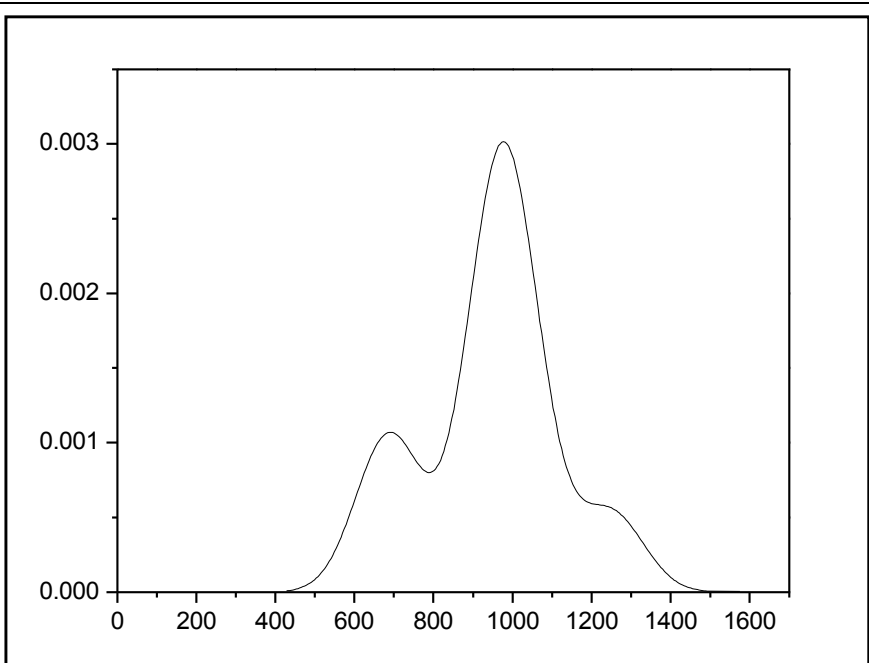


Figure 1b :
Results and
95% confidence intervals
(expanded uncertainty),
as reported by participants

$X_{ref} = 1017$ ppb
 $u_{ref} = 21.2$ ppb
 $\sigma_p = 162$ ppb

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

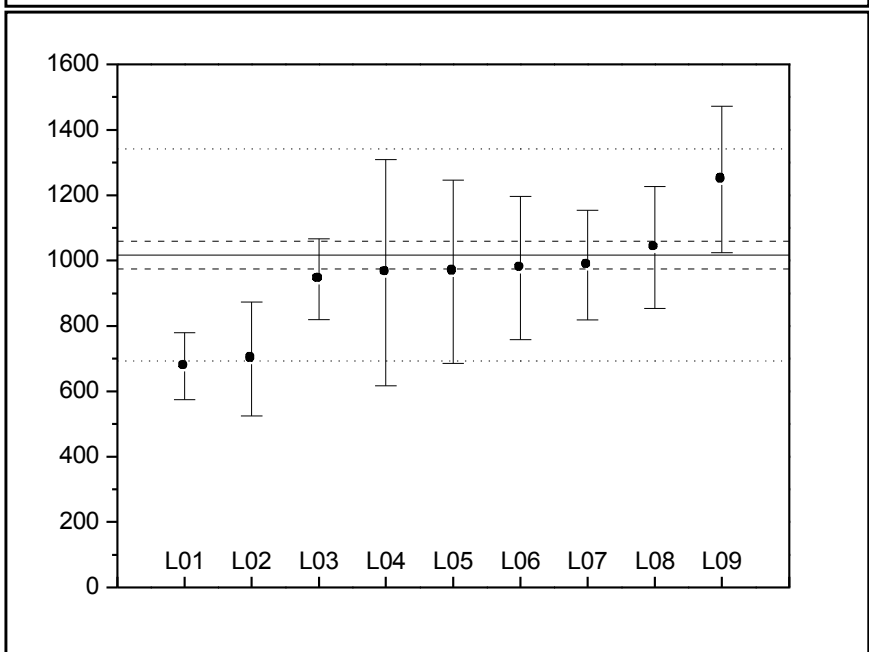
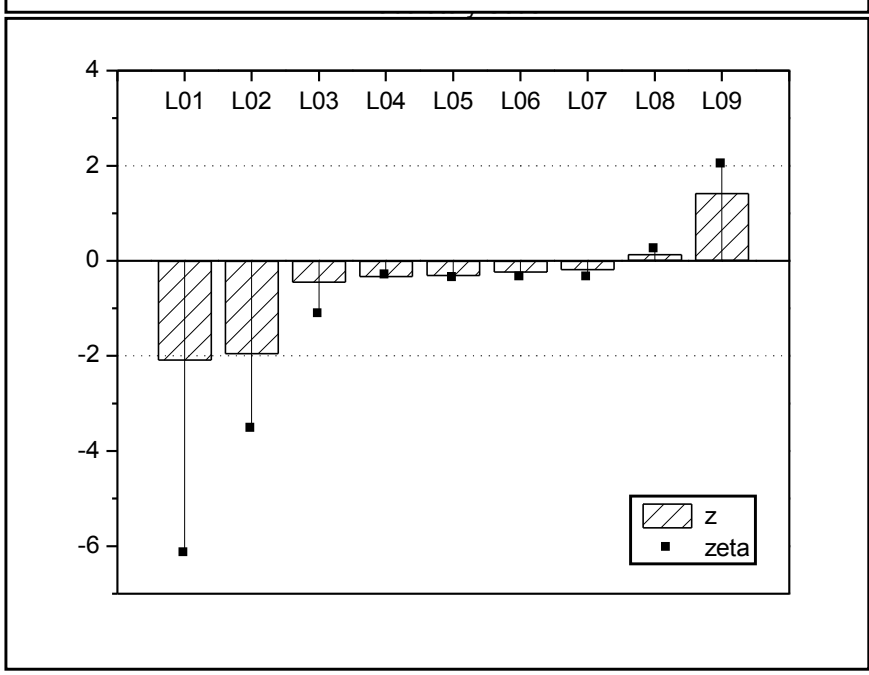


Figure 1c : z- and zeta-scores

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

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



Annexes

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

Organisation	Address	
CER Marloie	Rue du Point du Jour, 8	6900 Marloie
CERVA	Leuvensesteenweg, 17	3080 Tervuren
CRAW	rue de Liroux, 4	5030 Gembloux
FLVVT	Leuvensesteenweg, 17	3080 Tervuren
Fytolab	Technologiepark, 2/3	9052 Zwijnaarde
LABECCA	Ambachtsweg, 3	9820 Merelbeke
OLEOTEST	Lage weg, 427	2660 Antwerpen
SGS-Agrilab	Polderdijkweg, 16 Haven 407	2030 Antwerpen
SGS-IAC	Polderdijkweg, 16 Haven 407	2030 Antwerpen



C O D A - C E R V A
DÉPARTEMENTS QUALITÉ ET SÉCURITÉ DE LA CHAÎNE ALIMENTAIRE
LEUVENSESTEENWEG 17 - B 3080 TERVUREN - TÉL: 02/769.22.80 - FAX: 02/769.23.05

Ring-Test "Déoxynivalénol"

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. The sample is sent in his original packaging. The 55gr portion was 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 june2008. The testing method should be the laboratory routinely method used for DON determination. The tests should be finished at 30th of august the latest.

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, method description and uncertainty 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 do not forget to state your coverage factor.

Receipt form :

RING – TEST DEOXYNIVALENOL VAR 06/2008

Please send to : J.C.Motte , FAX n° 02/769.23.05

Annexe 4: receipt form

Name of the Laboratory :	
Name of the analyst :	

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

Date	stamp	Signature

Results form :

RING – TEST DEOXYNIVALENOL VAR 06/2008

SUBMIT RESULTS TO : jemot@var.fgov.be

DEADLINE :	30 th of august 2008 at 0.00h
Name of the Laboratory :	
Name of the analyst :	

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

	Yes	no
Recovery in the same matrix		

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

Datum :

Stamp :

Signature :