CODA-CERVA

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

Belgian National Reference Laboratory for Trace Elements in Food and Feed

NRL NATIONAL REFERENCE LABORATORIES

PT-2014-NRL-TE-FASFC

Determination of Cu, Zn, As, As_i, Cd and Pb in wine

Final report on the 2014 interlaboratory comparison organised by the National Reference Laboratory for Trace Elements in Food and Feed

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Dr ir Karlien Cheyns and Dr ir Nadia Waegeneers

Central office
Veterinary Operational Directions:
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 Operational Direction:
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

e-mail info@coda-cerva.be website : http://www.coda-cerva.be

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Summary

From the 1st of January 2008, the laboratory for Trace Elements in the Veterinary and Agrochemical Research Centre (CODA-CERVA), Tervuren, operates as National Reference Laboratory for Trace Elements in Feed and Food (NRL-TE). One of its core tasks is to organise interlaboratory comparisons (ILCs) among laboratories appointed by the Federal Agency for the Safety of the Food Chain. This report presents the results of the interlaboratory comparison organised by the NRL-TE which focused on the determination of trace elements in wine. The results from the ILC were treated in CODA-CERVA, Tervuren.

The 2014 ILC was obligatory for all laboratories approved for the analysis of heavy metals in foodstuff by the Federal Agency for the Safety of the Food Chain (FASFC). Nine laboratories registered for and participated in the exercise.

The test material used in this test was red wine. The wine was purchased in a local supermarket. As the natural concentrations of arsenic, inorganic arsenic, cadmium and lead were low, the material was spiked with these elements in the laboratory. Each participant received approximately 15 ml of test material.

Participants were invited to report the mean value and measurement uncertainty on their results for copper (Cu), zinc (Zn), arsenic (As), inorganic arsenic (As_i), cadmium (Cd) and lead (Pb).

The assigned values (x_a) and their uncertainty ($u(x_a)$) were determined as the consensus of participant's results. Standard deviations for proficiency assessment were calculated using the modified Horwitz equation. If less than seven data points were collected it was not possible to calculate any assigned value, in this case only an informal consensus value and informal z and ζ-scores are given.

Of the nine laboratories that registered for participation, nine submitted results for As and Cd, eight submitted results for Cu, Zn and Pb and four submitted results for As_i. All of the 44 (informal) z-scores that were calculated, were satisfactory. Of the 44 (informal) ζ-scores only one was questionable.

Introduction

Trace elements occur in varying amounts as natural elements in soils, plants and animals, and consequentially in food and feed. Concerning food and feed of plant origin, the characteristics of the soil on which the plants are grown have a considerable influence on the content of trace elements in the plant. The concentration of trace elements in plants is often correlated to the corresponding concentrations in the soil on which they were grown, but also soil texture, soil pH and soil organic matter content influence the trace element content in the plants. To ensure public health, maximum levels for trace elements in foodstuff have been laid down in the Commission Regulation (EC) N° 1881/2006. Regarding wine these maximum levels are 0.20 mg/kg for lead (harvested from 2001 onwards). There is currently no European legislation regarding cadmium, zinc or arsenic levels in wine. The discussion for regulation of maximum levels of arsenic and inorganic arsenic is ongoing at the European level. Copper levels are regulated in the Commission Regulation (EC) N° 396/2005 regarding maximum pesticide residues levels in food and feed of plant and animal origin. There is no legislation for copper in wine but copper in wine grapes is limited to 50 mg/kg.

The scope of this ILC was to test the competence of the participating laboratories to determine the total mass fraction of Cu, Zn, As, As_i, Cd and Pb in wine.

Time frame, test material and instructions to participants

Invitation letters to this ILC were sent to participants in April (Annex 1). The 2014 ILC was obligatory for all laboratories approved for the analysis of heavy metals in foodstuff by the Federal Agency for the Safety of the Food Chain (FASFC). Nine laboratories, which were approved for these foodstuffs, registered for and participated in the exercise. The samples were dispatched to the participants by the beginning of June 2014. Reporting deadline was the first of July.

This year the test material was a sample of red wine. The wine was purchased in a local supermarket. As the natural concentrations of As(i), Cd and Pb were low, the material was spiked in the laboratory. This was done by adding metal-salt solutions of $Cd(NO_3)_2$, $Pb(NO_3)_2$ and a mixture of Na_2HAsO_4 and dimethylarsinic acid (DMA). The material was shaken well and divided in small portions of about 15 mL.

The homogeneity of the test material was tested following the recommended procedure according to IUPAC [1]. All the trace elements appeared to be homogeneously distributed in the samples (Annex 2). Each participant received one bottle of test material, an accompanying letter (Annex 3) with instructions on sample handling and reporting (Annex 4), a form that had to be sent after receipt of the sample to confirm its arrival (Annex 5) and a reporting form (Annex 6).

Participants were instructed to store the material in a dark place at 4 °C until analysis. Before starting the analyses, the sample had to be re-homogenized by shaking for about 30 seconds. The procedure followed for the exercise, had to be as close as possible to the method used by the participant in routine sample analysis. Nevertheless participants were instructed to perform three independent measurements per parameter and to report measurement uncertainty.

A questionnaire was attached to the reporting form. The questionnaire was intended to provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in Annex 6.

Laboratory codes were given randomly and communicated confidentially to the corresponding participant.

Assigned values

The assigned values for the different trace elements in the red wine sample were determined as the consensus of participant's results [1]. The major advantages of consensus values are the straightforward calculation and the fact that none of the participants is accorded higher status. The disadvantages are that the consensus values are not independent of the participant's results and, especially in the current case with nine participants, that the uncertainty on the consensus (identified as the standard error) may be high and the information content of the z-scores will be correspondingly reduced.

The robust statistic approach is a convenient modern method of handling results when they are expected to follow a near-normal distribution and it is suspected that they include a small proportion of outliers. There are many different robust estimators of mean and standard deviation [2]. The median and MAD (median absolute difference) were chosen here as robust estimators.

The modified Horwitz equation was used to establish the standard deviation for proficiency testing (σ_p) [1][3]. It is an exponential relationship between the variability of chemical measurements and concentration. The Horwitz value is widely recognized as a fitness-for-purpose criterion in proficiency testing.

The Kernel density estimate gives a good estimate of the population density function without making any assumptions that it is a normal distribution. The Kernel distribution plots were obtained using a software tool developed by AMC [4].

The scheme that was followed to estimate the consensus and its uncertainty is outlined below [1], in the current case steps (a), (d) and (e) were not needed:

a) Results that were identifiable invalid or extreme outliers were excluded.

b) A visual presentation of the remaining results was examined. It was checked whether the distribution was apparently unimodal and roughly symmetric, possible outliers aside. If so $\Rightarrow$ c); else $\Rightarrow$ d).

c) The robust mean $\hat{\mu}_{rob}$ and standard deviation $\hat{\sigma}_{rob}$ of the n results were calculated as $\hat{\mu}_{rob}$ = median and $\hat{\sigma}_{rob}$ = 1.4826*MAD. If $\hat{\sigma}_{rob}$ was less than about $1.2\sigma_p$, then $\hat{\mu}_{rob}$ was used as the assigned value x_a and $\hat{\sigma}_{rob}/\sqrt{n}$ as its standard uncertainty $u(x_a)$.

d) A Kernel density estimate of the distribution was made using normal kernels with a bandwith h of $0.75\sigma_p$. If this resulted in a unimodal and roughly symmetric kernel density, and the mode and median were nearly coincident, then $\hat{\mu}_{rob}$ was used as the assigned value and $\hat{\sigma}_{rob}/\sqrt{n}$ as its standard uncertainty; else $\Rightarrow$ e).

e) *If the minor mode could be safely attributed to an outlying result, then* $\hat{\mu}_{rob}$ *was still used as the assigned value and* $\hat{\sigma}_{rob}/\sqrt{n}$ *as its standard uncertainty; else no consensus value could be derived.*

The consensus values, their standard uncertainty and some other statistical parameters are summarised in Table 1.

Table 1 : Summary of statistical parameters for the test material.

	Cu * mg/kg	Zn mg/kg	As mg/kg	As_i * mg/kg	Cd mg/kg	Pb mg/kg
N	6	8	9	4	9	8
Mean	0.198	1.64	0.121	0.067	0.102	0.132
SD	0.019	0.17	0.010	0.009	0.008	0.007
Robust mean (median)	0.198	1.65	0.121	0.066	0.101	0.133
Robust SD	0.016	0.17	0.006	0.010	0.007	0.008
Assigned value x_a	**0.198**	**1.65**	**0.121**	**0.066**	**0.101**	**0.133**
Standard uncertainty of the assigned value $u(x_a)$	**0.007**	**0.06**	**0.003**	**0.005**	**0.002**	**0.003**
σ_p	0.043	0.36	0.027	0.015	0.022	0.029

Assigned value x_a: median of the reported results; σ_p: standard deviation for proficiency assessment;
*Informal consensus values.

Scores and evaluation criteria

Individual laboratory performances are expressed in terms of z-scores and ζ-scores in accordance with ISO 135283 and the International Harmonised Protocol [1].

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$

where:

x_{lab} is the mean of the individual measurement results as reported by the participant

x_a is the assigned value

σ_p is the standard deviation for proficiency assessment

$u(x_a)$ is the standard uncertainty for the assigned value

$u(x_{lab})$ is the reported standard uncertainty on the reported value x_{lab}. When no uncertainty was reported by the laboratory, it was set to zero.

The z-score compares the participant's deviation from the reference value with the standard deviation accepted for the proficiency test, σ_p. Should participants feel that these σ values are not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements.

The z-score can be interpreted as:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

The ζ-score states if the laboratory result agrees with the assigned value within the uncertainty claimed by this laboratory (taking due account of the uncertainty on the reference value itself). The interpretation of the ζ-score is similar to the interpretation of the z-score.

$\|\zeta\| \leq 2$	satisfactory result
$2 < \|\zeta\| \leq 3$	questionable result
$\|\zeta\| > 3$	unsatisfactory result

Per trace element, a set of figures is provided. Each set includes (a) the Kernel density plot (except for Cu and As_i) (b) the individual mean values with their reported uncertainty, and (c) the z- and ζ-scores. The solid line represents the assigned value, the dashed lines delimit the reference interval ($x_a \pm 2u(x_a)$) and the dotted lines delimit the target interval ($x_a \pm 2\sigma_p$).

Results

Copper (Cu)

x_a = **0.198** ± 0.014 µg/kg (k = 2)

Nine laboratories submitted results for Cu concentrations. Two laboratories could not produce results above their limit of quantification. The relative standard deviation of the values of the other six laboratories was less than 15%. Therefore, the median of these four results was used as informal assigned value. The six laboratories obtained satisfactory informal z-scores for Cu against the standard deviation accepted for the proficiency test (Table 2; Figure 1). These laboratories did also obtain good informal ζ-scores against their stated measurement uncertainty. No quantification limits were reported that were lower than the corresponding x_a-3 $u(x_a)$ value, so those statements are satisfactory.

Table 2 : values reported for Cu (mg/kg) by the participants and informal scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 1 (mg kg^{-1})	Result 1 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	Informal z-scores	Informal ζ-scores
1	0.20	0.202	0.204	0.203	0.061	0.1	0.2
2							
3	0.181	0.175	0.180	0.179	0.035	-0.4	-1.0
4	<1	<1	<1				
5	0.179	0.190	0.175	0.181	0.035	-0.4	-0.9
6	0.186	0.196	0.195	0.192	0.048	-0.1	-0.2
7	0.237	0.237	0.223	0.232	0.045	0.8	1.5
8	0.206	0.203	0.201	0.203	0.045	0.1	0.2
9	<0.318	<0.35	<0.364				

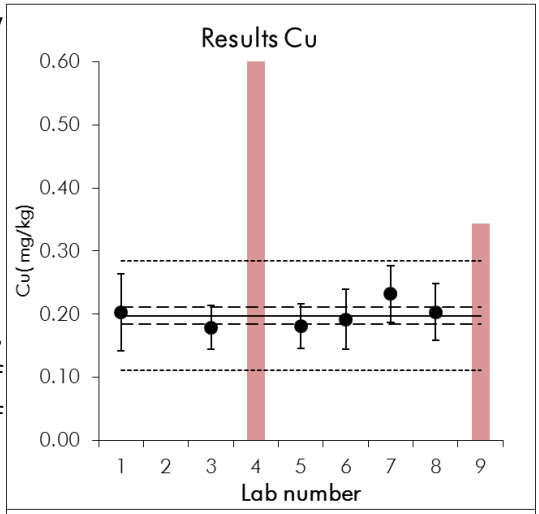
Figure 1 : (b) Results with expanded uncertainty for Cu, as reported by the participants

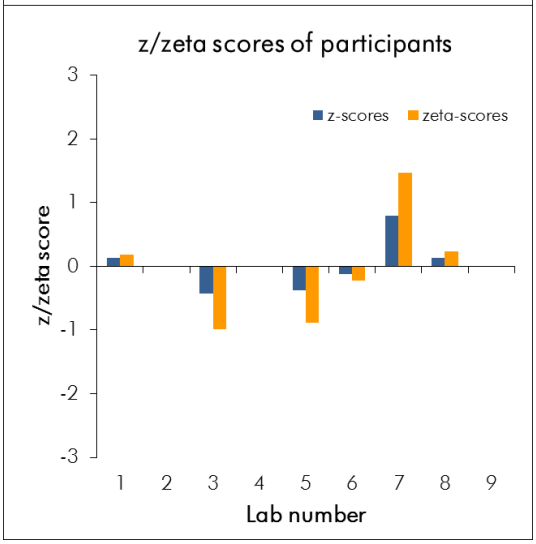
x_a = 0.198 mg kg^{-1}

$U(x_a)$ = 0.014 mg kg^{-1}

σ_p = 0.043 mg kg^{-1}

(dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs), the vertical ax is cut off at 0.6.





(c) informal z and ζ-scores

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$

Zinc (Zn)

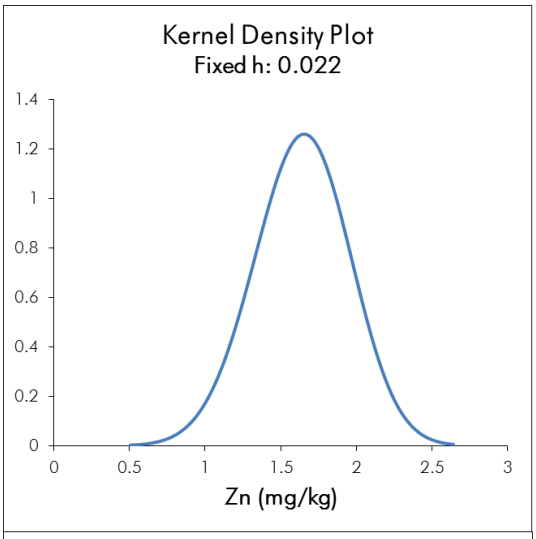
x_a = **1.64** ± 0.12 mg/kg (k = 2)

Eight laboratories submitted results for Zn concentrations. All laboratories obtained satisfactory z-scores for Zn against the standard deviation accepted for the proficiency test (Table 3; Figure 2). All but one laboratory obtained satisfactory ζ-scores against their stated measurement uncertainty. Lab L08 obtained a questionable ζ-score.

Table 3 : values reported for Zn (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	1.752	1.809	1.918	1.826	0.548	0.5	0.6
2							
3	1.560	1.490	1.530	1.530	0.310	-0.3	-0.7
4	1.745	1.730	1.695	1.723		0.2	1.2
5	1.584	1.593	1.527	1.568	0.309	-0.2	-0.5
6	1.650	1.550	1.540	1.580	0.395	-0.2	-0.3
7	1.806	1.767	1.693	1.755	0.346	0.3	0.6
8	1.142	1.370	1.451	1.321	0.251	-0.9	-2.4
9	1.810	1.810	1.770	1.800	0.130	0.4	1.7

Figure 2 : (a) Kernel density plot for Zn results



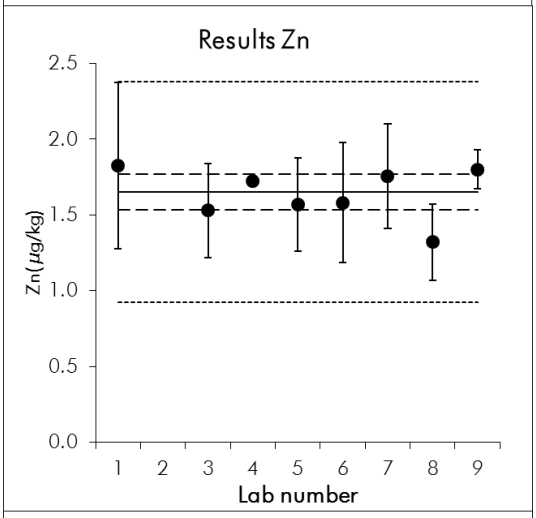
(b) Results with expanded uncertainty for Zn, as reported by the participants

x_a = 1.65 mg kg^{-1}

$U(x_a)$ = 0.12 mg kg^{-1}

σ_p = 0.36 mg kg^{-1}

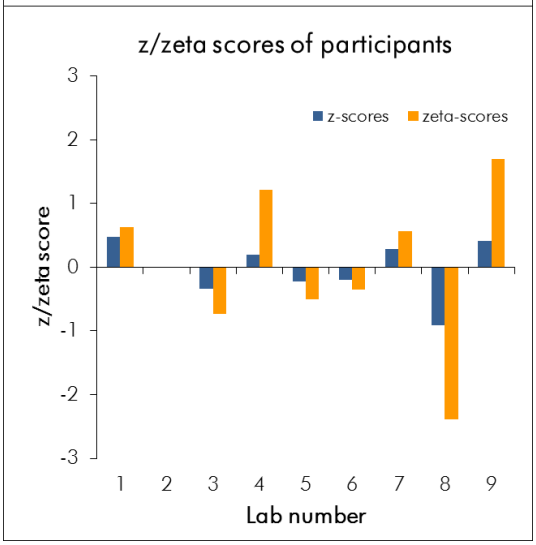
(dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs)



(c) z and ζ-scores

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$



Arsenic (As)

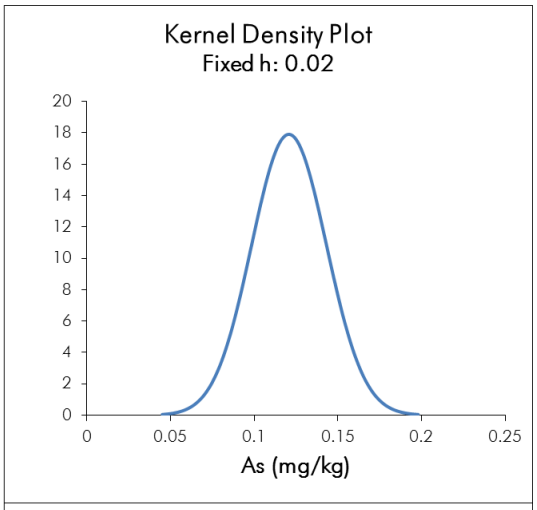
x_a = **0.121** ± 0.006 mg/kg (k = 2)

Nine laboratories submitted results for As concentrations. All laboratories obtained satisfactory z-scores for As against the standard deviation accepted for the proficiency test (Table 4; Figure 3). All laboratories obtained satisfactory ζ-scores against their stated measurement uncertainty.

Table 4 : values reported for As (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.135	0.130	0.130	0.132	0.030	0.4	0.7
2	0.126	0.117	0.112	0.118	0.046	-0.1	-0.1
3	0.109	0.103	0.103	0.105	0.029	-0.6	-1.1
4	0.120	0.119	0.125	0.122	0.040	0.0	0.0
5	0.122	0.126	0.116	0.121	0.029	0.0	0.0
6	0.112	0.113	0.108	0.111	0.028	-0.4	-0.7
7	0.141	0.141	0.133	0.138	0.033	0.6	1.0
8	0.102	0.127		0.115	0.023	-0.2	-0.5
9	0.124	0.124	0.131	0.126	0.006	0.2	1.2

Figure 3 : (a) Kernel density plot for As results



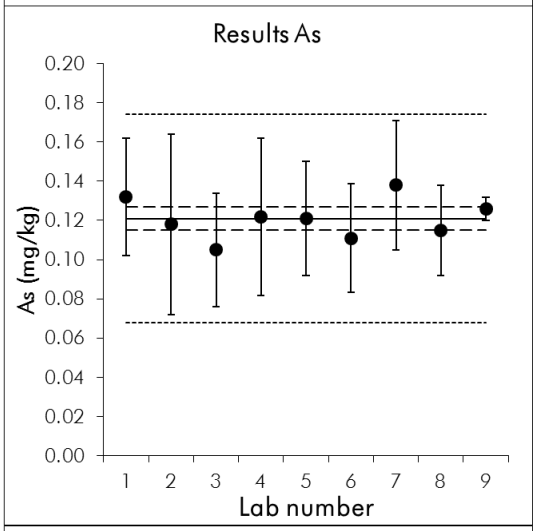
(b) Results with expanded uncertainty for As, as reported by the participants

x_a = 0.121 mg kg^{-1}

$U(x_a)$ = 0.006 mg kg^{-1}

σ_p = 0.027 mg kg^{-1}

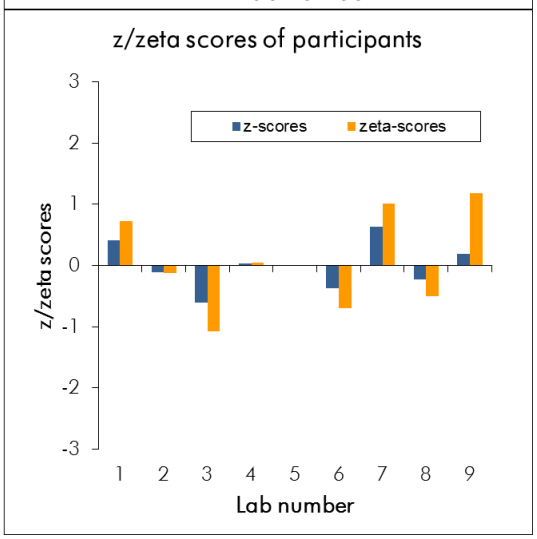
(dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs)



(c) z and ζ-scores

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$



Inorganic arsenic (As_i)

x_a = **0.066** ± 0.010 mg/kg (k = 2)

Four laboratories submitted results for As_i concentrations. The relative standard deviation of the values of the results was less than 15%. Therefore, the median of these four results was used as informal consensus value. The four laboratories obtained satisfactory informal z-scores for As against the standard deviation accepted for the proficiency test (Table 5; Figure 4). These laboratories did also obtain good informal ζ-scores against their stated measurement uncertainty.

Table 5 : values reported for As_i (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.075	0.077	0.079	0.077	0.030	0.8	0.7
2							
3	0.057	0.056	0.055	0.057	0.029	-0.6	-0.6
4	0.055	0.055	0.074	0.061	0.040	-0.3	-0.2
5							
6							
7	0.073	0.070		0.071	0.029	0.3	0.3
8							
9							

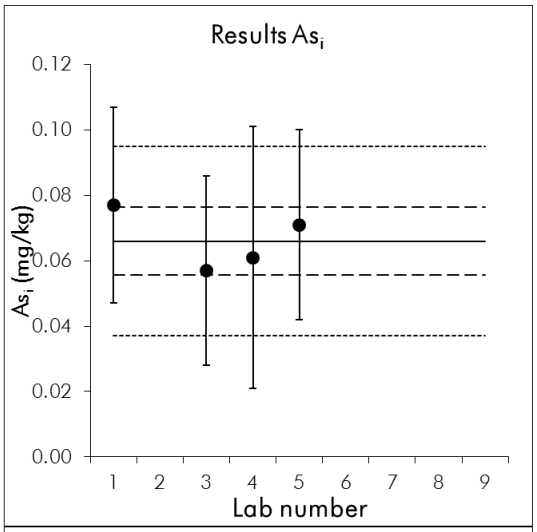
Figure 4 : (a) Results with expanded uncertainty for As, as reported by the participants

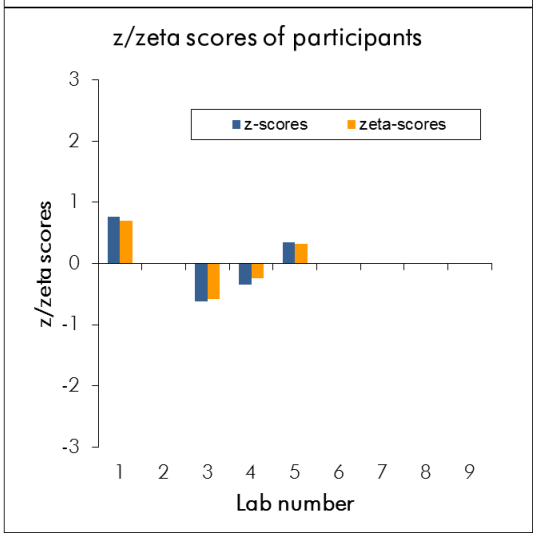
x_a = 0.066 mg kg^{-1}

$U(x_a)$ = 0.010 mg kg^{-1}

σ_p = 0.015 mg kg^{-1}

(dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs)





(b) z and ζ-scores

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$

Cadmium (Cd)

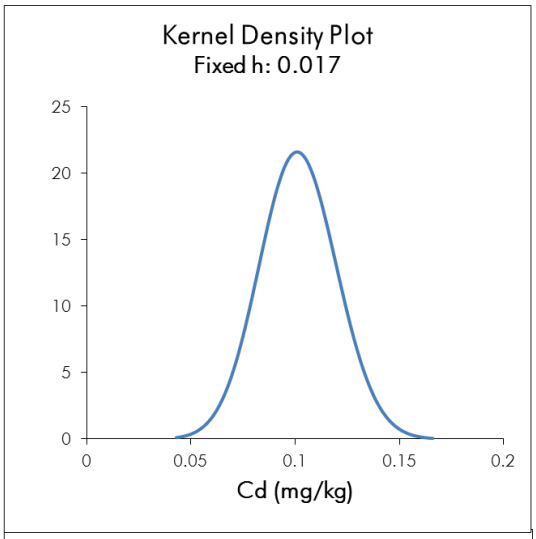
x_a = **0.101** ± 0.004 mg/kg (k = 2)

Nine laboratories submitted results for Cd concentrations. All laboratories obtained satisfactory z-scores for Cd against the standard deviation accepted for the proficiency test (Table 6; Figure 5). All laboratories obtained satisfactory ζ-scores against their stated measurement uncertainty.

Table 6 : values reported for Cd (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.102	0.102	0.100	0.101	0.025	0.0	0.0
2	0.099	0.101	0.104	0.101	0.027	0.0	0.0
3	0.097	0.092	0.092	0.094	0.035	-0.3	-0.4
4	0.101	0.101	0.102	0.101	0.026	0.0	0.0
5	0.098	0.101	0.095	0.098	0.013	-0.1	-0.4
6	0.096	0.096	0.094	0.095	0.024	-0.3	-0.5
7	0.118	0.117	0.111	0.115	0.015	0.6	1.8
8	0.094	0.095	0.100	0.096	0.020	-0.2	-0.5
9	0.106	0.106	0.126	0.113	0.018	0.5	1.3

Figure 5 : (a) Kernel density plot for Cd results



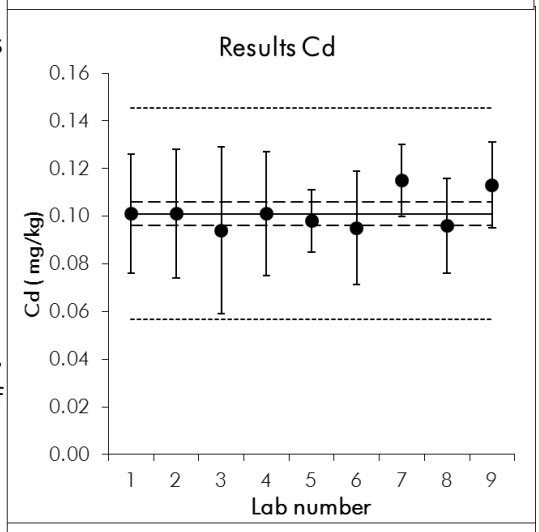
(b) Results with expanded uncertainty for Cd, as reported by the participants

x_a = 0.101 mg kg^{-1}

$U(x_a)$ = 0.004 mg kg^{-1}

σ_p = 0.022 mg kg^{-1}

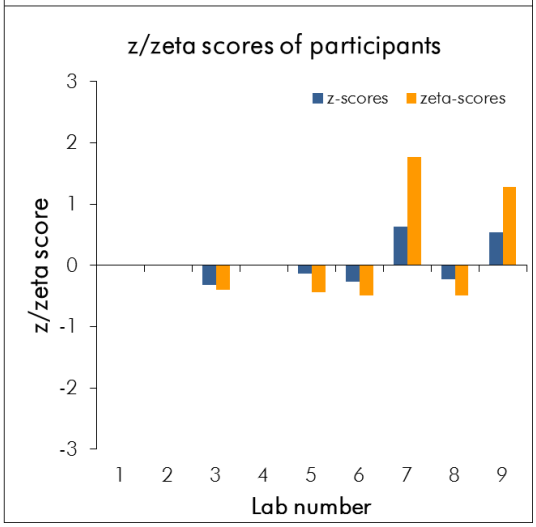
(dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs)



(c) z and ζ-scores

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$



Lead (Pb)

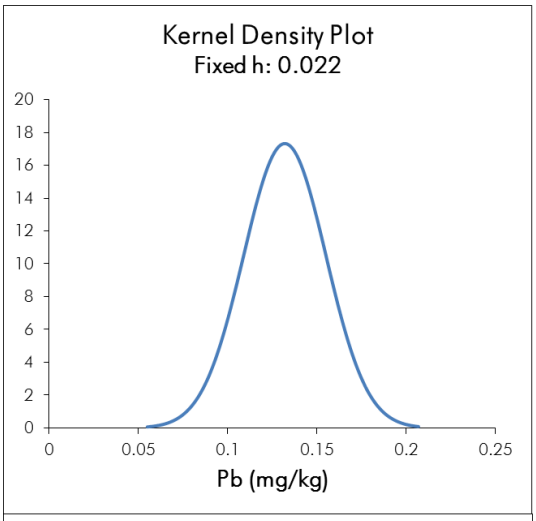
x_a = **0.133** ± 0.006 mg/kg (k = 2)

Eight laboratories submitted results for Pb concentrations. All laboratories obtained satisfactory z-scores for Pb against the standard deviation accepted for the proficiency test (Table 7; Figure 6). All laboratories obtained satisfactory ζ-scores against their stated measurement uncertainty.

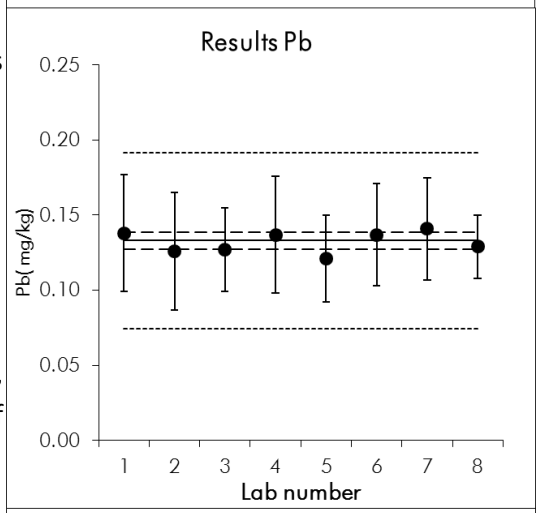
Table 7 : values reported for Pb (mg/kg) by the participants and scores calculated by the organizer

Lab code	Result 1 (mg kg^{-1})	Result 2 (mg kg^{-1})	Result 3 (mg kg^{-1})	Mean (mg kg^{-1})	Extended uncertainty (k = 2) (u_{lab}; mg kg^{-1})	z-scores	ζ-scores
1	0.140	0.134	0.137	0.138	0.039	0.2	0.3
2	0.124	0.112	0.141	0.126	0.039	-0.2	-0.4
3	0.130	0.124	0.127	0.127	0.028	-0.2	-0.4
4	0.139	0.137	0.136	0.137	0.039	0.1	0.2
5	0.123	0.124	0.118	0.121	0.029	-0.4	-0.8
6	0.137	0.137	0.137	0.137	0.034	0.1	0.2
7	0.145	0.142	0.136	0.141	0.034	0.3	0.5
8	0.128	0.145	0.115	0.129	0.021	-0.1	-0.4
9							

Figure 6 : (a) Kernel density plot for Pb results



(b) Results with expanded uncertainty for Pb, as reported by the participants



x_a = 0.133 mg kg^{-1}

$U(x_a)$ = 0.006 mg kg^{-1}

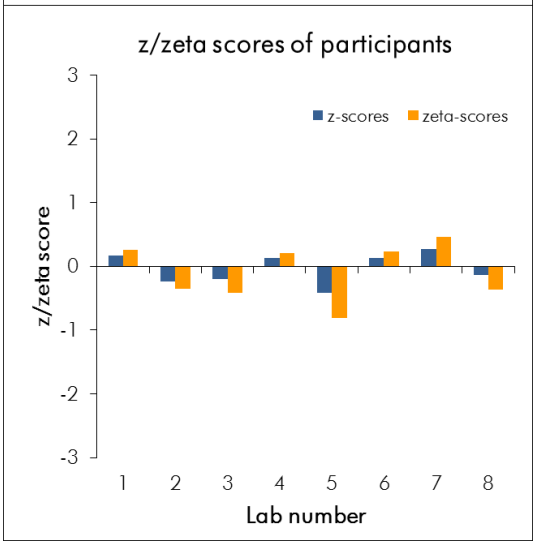
σ_p = 0.029 mg kg^{-1}

(dashed lines: $x_a \pm 2\ u(x_a)$, dotted lines: $x_a \pm 2\ \sigma_p$, red bars represent the limits of quantification of the corresponding labs)

(c) z and ζ-scores

$$z = \frac{x_{lab} - x_a}{\sigma_p}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u^2(x_a) + u^2(x_{lab})}}$$



Discussion

All nine laboratories that registered for participation did submit results. All nine submitted results for As and Cd, eight submitted results for Cu, Zn and Pb and four submitted results for inorganic As. In case of Cu, the concentration was lower than the limit of quantification for two laboratories. From the results, values reported as “less than” were not included in the evaluation.

The most commonly used technique for the analysing of trace element concentrations was ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). Only two exceptions were noticed: one lab uses INAA (Instrumental Neutral Activation Analysis) and one lab HG-AAS (Hydride Generation-Atomic Absorption Spectroscopy). For Cu and/or Zn some laboratories used ICP-OES. The number of participants per technique was too small to make inferences on the performance of the different techniques.

For the first time inorganic Arsenic was included in the ILC. The four participating laboratories had similar results and the relative standard deviation of these results was only 14 %. All of the As_i measurements were done with ICP-MS, however information about the species separation method is not given by all participants.

All of the 44 z-scores that were calculated were satisfactory. Of the 44 ζ-scores, 43 were satisfactory and one was questionable. No quantification limits were reported that were lower than the corresponding x_a-3 $u(x_a)$ value, so those statements are satisfactory.

Bibliography

[1] M. Thompson, S. L. R. Ellison, and R. Wood, "The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report)," *Pure and Applied Chemistry*, vol. 78, no. 1, pp. 145–196, 2006.

[2] AMC, "Robust statistics: a method of coping with outliers," *AMC technical brief*, no. 6, 2001.

[3] M. Thompson, "The amazing Horwitz function," *AMC Technical Brief*, no. 17, 2004.

[4] M. Thompson, "Representing data distributions with kernel density estimates," *AMC Technical Brief*, no. 4, pp. 1–2, 2006.

Annexes

Annex 1: Invitation letter to laboratories

CODA-CERVA

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

Dear colleague,

It is our pleasure to invite you to participate in the proficiency test (PT) for the detection of trace elements in food, organized by the National Reference Laboratory (NRL) for trace elements in food and feed at CODA-CERVA. The goal of the PT is to determine the performance of individual laboratories for specific tests. The PT is organised according to the ISO/IEC 17043 norm: 2010 Conformity assessment – General requirements for proficiency testing.

The following PT will be organized by the NRL for trace elements in food and feed in 2014 for the laboratories involved in the official control program of the Federal Agency for the Safety of the Food Chain (FASFC) and other interested laboratories:

PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine"

- Closing date for the inscription: 18th of April 2014 (week 16)
- Shipment of the samples: 2nd of June 2014 (week 23)
- Submission of the test results: 20th of June 2014 (week 25)
- Draft report: 8th of September 2014 (week 37)
- Final report: 6th of October 2014 (week 41)

If your laboratory is approved by the FASFC for trace elements in foodstuffs, participation to the PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine" is mandatory for all accredited elements and the costs for PT-2014-NRL-TE-FASFC (€ 218.54) will be billed directly by the Federal Agency for the Safety of the Food Chain (FASFC). The individual results of the laboratories approved by the FASFC will be disclosed to the FASFC.

If your laboratory is not approved by the FASFC for trace elements in foodstuffs, participation to the PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine" is voluntary and the costs for PT-2014-NRL-TE-FASFC, € 218.54 + shipment costs, will be billed by CODA-CERVA. The results will not be disclosed to the FASFC.

You can receive more information about our PT programme by contacting karlien.cheyns@coda-cerva.be

We hope you will find this a useful tool to support your laboratory's Quality Assurance system and look forward to receiving your registration before **the 18th of April 2014**. If you are not the correct contact person for this message or if you know other colleagues that might be interested, please feel free to forward this invitation to your own colleagues or colleagues from other institutes.

If you would no longer like to receive this invitation, please send us a reply and we will remove you from our contact list.

Kind regards,

Dr ir Karlien Cheyns and Dr ir Nadia Waegeneers
Belgian National Reference Laboratory for Trace Elements in Food and Feed
CODA-CERVA

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

Central office
Veterinary Operational Directions:
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 Operational Direction:
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

e-mail info@coda-cerva.be website : http://www.coda-cerva.be

.be

Invitation letter PT-2014-NRL-TE.docx

Annex 2: Results of the homogeneity studies

	Cu	Zn	As	Cd	Pb
Cochran test for variance outliers					
Cochran test statistic	0.668	0.650	0.764	0.678	0.539
Critical (99%)	0.718	0.718	0.718	0.718	0.718
Cochran < critical	use complete dataset	use complete dataset	Remove outlier	use complete dataset	use complete dataset
Test for sufficient homogeneity					
S_{an}^2	1288	36	5	10	25
S_{sam}^2	1601	36	14.4	2	4
σ_{all}^2	6389	175	94	56	85
F1	1.88	1.88	1.94	1.88	1.88
F2	1.01	1.01	1.11	1.01	1.01
Critical	13312	533	187	115	185
S_{sam}^2 **< critical?**	accept	accept	accept	accept	accept

Annex 3: Letter accompanying the sample

CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

May 28th 2014

Dear colleague,

Following your subscription for the proficiency test (PT-2014-NRL-TE-FASFC) for the detection of trace elements in food, we ship you the PT sample.

Enclosed you can find the **instructions to the participants** with a **reporting form**. In addition, a **receipt form** is added, please retour this by fax (+32 2 7692305) or e-mail (karlien.cheyns@coda-cerva.be). The time schedule of the PT is given below:

PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine"

- Closing date for the inscription: 18th of April 2014 (week 16)
- Shipment of the samples: 2nd of June 2014 (week 23)
- Submission of the test results: 20th of June 2014 (week 25)
- Draft report: 8th of September 2014 (week 37)
- Final report: 6th of October 2014 (week 41)

We expect the results of the analysis the latest by the end of week 25 (the 20th of June).

We would like to remind you that if your laboratory is approved by the FASFC for trace elements in foodstuffs, participation to the PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine" is mandatory for all accredited elements and the costs for PT-2013-NRL-TE-FASFC (€ 218.54) will be billed directly by the Federal Agency for the Safety of the Food Chain (FASFC). The individual results of the laboratories approved by the FASFC will be disclosed to the FASFC. If your laboratory is not approved by the FASFC for trace elements in foodstuffs, participation to the PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine" is voluntary and the costs for PT-2014-NRL-TE-FASFC, € 218.54 + shipment costs, will be billed by CODA-CERVA. The results will not be disclosed to the FASFC.

For any information about our PT programme you can contact karlien.cheyns@coda-cerva.be

Kind regards,

Dr ir Karlien Cheyns and Dr ir Nadia Waegeneers

Belgian National Reference Laboratory for Trace Elements in Food and Feed
CODA-CERVA

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

Central office
Veterinary Operational Directions:
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 Operational Direction:
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

e-mail info@coda-cerva.be website : http://www.coda-cerva.be .be

Shipment PT-NRL-TE_2014.doc

Annex 4: Instructions to participants

CODA-CERVA
CENTRUM VOOR ONDERZOEK IN DIERGENEESKUNDE EN AGROCHEMIE
CENTRE D'ETUDE ET DE RECHERCHES VÉTÉRINAIRES ET AGROCHIMIQUES

PRO/2.5/06/DOC03/V02 : INSTRUCTIONS TO THE PARTICIPANTS
PRO/2.5/06/DOC03/V02 : INSTRUCTIES AAN DE DEELNEMERS
PRO/2.5/06/DOC03/V02 : INSTRUCTIONS AUX PARTICIPANTS

Type of proficiency test / Type proficiency test / Type d'essai d'aptitude :

PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine"

Analyte(s) / Analyt(en) / Analyte(s) :

As, As_i , Cd, Pb, Cu , Zn,

Matrix(-ces) / Matrix(-ces) / Matrice(s) :

Red wine

Number of materials sent / Aantal verstuurde materialen / Nombre de matériaux envoyés :

One small container, about 15 mL

Storage method / Wijze van bewaring / Mode de conservation :

Cool (4°C), dark conservation

Data to be sent and to whom / Gegevens die moeten opgestuurd worden en aan wie / Données à envoyer et à qui :

See 'results reporting form', to be transmitted to Karlien Cheyns, preferably by e-mail: karlien.cheyns@coda-cerva.be (an electronic version of the reporting form will be sent by e-mail). Fax: +32 2 769 2305; Address: Coda-Cerva, Leuvensesteenweg 17, 3080 Tervuren

Deadline for sending the results to the OD-CSF / Datum (deadline) waarop de resultaten moeten opgestuurd worden naar de OD-CVV / Date (deadline) à laquelle les résultats doivent être envoyés à la DO-SCA :

20/06/2014

Specific instructions / Specifieke Intructies / Instructions spécifiques :

- *Shake the sample manually during 30 seconds before analysis*
- *Follow as close as possible the analysis method you use in routine sample analysis*
- *The analysis should be performed in triplicate*
- *Report the extended uncertainty*

Reminder : for the Belgian official control labs, the results are communicated to the FASFC (FAVV-AFSCA)
Herinnering : Voor de Belgische erkende labo's worden de resultaten aan het FAVV meegedeeld
Rappel : Pour les laboratoires belges agréés, les résultats sont communiqués à l'AFSCA

Annex 5: Materials receipt form

CODA-CERVA
CENTRUM VOOR ONDERZOEK IN DIERGENEESKUNDE EN AGROCHEMIE
CENTRE D'ETUDE ET DE RECHERCHES VÉTÉRINAIRES ET AGROCHIMIQUES

PRO/2.5/06/DOC04/V02:

PROFICIENCY TESTING MATERIALS RECEIPT FORM
FORMULIER VAN BEVESTIGING VAN ONTVANGST VAN HET MATERIAAL
FORMULAIRE DE CONFIRMATION DE RÉCEPTION DU MATÉRIEL

NAME ORGANISATION (LAB) / NAAM ORGANISATIE (LABO) / NOM ORGANISATION (LABO) :

CONTACT PERSON / CONTACTPERSOON / PERSONNE DE CONTACT :

TEL : FAX :

E-MAIL :

DATE OF THE RECEIPT / DATUM ONTVANGST VAN HET MATERIAAL / DATE DE RECEPTION DU MATERIEL :

STATE OF MATERIALS RECEIVED / STAAT BIJ ONTVANGST / ETAT A LA RECEPTION :

O GOOD / GOED / BON

O OPEN / OPEN / OUVERT

O BAD (specify) / SLECHT (specificeren) / MAUVAIS (à préciser) :

REMARKS / OPMERKINGEN / REMARQUES :

DATE / DATUM / DATE : SIGNATURE / HANDTEKENING / SIGNATURE :

Annex 6: Reporting form and questionnaire

CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

May 28th 2014

National Reference Laboratory for Trace Elements in Food and Feed

PT-2014-NRL-TE-FASFC "Determination of As, As_i, Cd, Pb, Cu and Zn in wine"

Results reporting form
Trace elements in red wine June 2014

Lab code: .....

1. **Does your laboratory carry out this type of analysis (as regards the parameters, matrix and methods) on a routine basis?**

   O Yes
   O No

2. **Which (other) matrices are analysed in your laboratory on a routine basis?**

   O Food of plant origin

   O Food of animal origin

   O Dairy products

   O Alcoholic beverages

   O Non-alcoholic beverages

   O Feed

   O Other: .....

3. **Is your laboratory interested in a particular matrix for future PT's?**

   .....

4. **Is your laboratory interested in the incorporation of inorganic arsenic in future PT's?**

   O Yes
   O No

CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

Lab Code: .....

Element	Technique used	Units	Replicate 1	Replicate 2	Replicate 3	Mean value	Extended uncertainty (k=2)
As		mg/kg					
As_i		mg/kg					
Cd		mg/kg					
Pb		mg/kg					
Cu		mg/kg					
Zn		mg/kg					