CCQM-K138
Determination of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs) in Dried Fig

Key Comparison
Track C

Final Report
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SUMMARY

The presence of any aflatoxin contamination in exported figs needs to be monitored and measured through reliable and traceable methods, which require pure and matrix certified reference materials. On the other hand, certified reference materials (CRM) for determination of aflatoxins in dried fig are not yet available. Moreover, there is a lack of CRMs to be used in routine testing laboratories for method validation and quality control. The routine testing laboratories, participating in commercial proficiency testing (PT) programs, use the results available from consensus values to evaluate the performance of the participating laboratories, rather than metrologically traceable assigned values. This study initially proposed as a key comparison and presented at the EURAMET TC-MC SCOA meeting in Malta in 2015 and subsequently at the CCQM OAWG meeting in April 2015, proposes a CRM candidate for determination of levels of aflatoxins B_1, B_2, G_1, G_2 and their total in dried fig [1-4]. Evidence of successful participation in formal, relevant international comparisons are needed to document measurement capability claims (CMCs) made by national metrology institutes (NMIs) and designated institutes (DIs).

In total nine NMI/DI participated in the Track C Key Comparison CCQM-K138 Determination of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs) in Dried Fig. Participants were requested to evaluate the mass fractions expressed in ng/g units, of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxin in a dried food matrix, dried fig. The CCQM-K138 results for the determination of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) are ranging from 5.17 to 7.27 ng/g with an %RSD of 10.47 for AFB_1, ranging from 0.60 to 0.871 ng/g with an %RSD of 11.69 for AFB_2, ranging from 1.98 to 2.6 ng/g with an %RSD of 10.36 for AFG_1, ranging from 0.06 to 0.32 ng/g with an %RSD of 35.6 for AFG_2, and ranging from 8.29 to 10.31 ng/g with an %RSD of 7.69 for Total AFs. All participants based their analyses on LC-MS/MS, HPLC-FLD, HR-LC/MS, and IDMS. Brief descriptions of the analytical methods used by the participants, including sample preparation, analytical technique, calibrants, and quantification approach are summarized in Appendix F. Linear Pool was used to assign the Key Comparison Reference Values (KCRVs) for B_1, B_2, G_1, G_2 and total aflatoxins. Due to the traceability requirements for the calibrants not being met, results of KEBS, INTI, VNIIM and BAM were excluded from KCRV determination.

Successful participation in CCQM-K138 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having high polarity ($pK_{ow} > -2$), in mass fraction range from 0.05 ng/g to 500 ng/g in dried food matrices.

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ACRONYMS

BAM	Bundesanstalt fuer Materialforschung und –pruefung, DI: Germany
CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CMC	Calibration and Measurement Capability
CRM	certified reference material
CV	coefficient of variation, expressed in %: CV = 100·s/$\bar{x}$
DI	designated institute
DoE	degrees of equivalence
EXHM	Chemical Metrology Laboratory, DI: Greece
GLHK	Government Laboratory, Hong Kong, DI: Hong Kong
HPLC-DAD	high pressure liquid chromatography with diode array detection
LC-HRMS	liquid chromatography with high-resolution mass spectrometry detection
LC-MS	liquid chromatography with mass spectrometry detection
LC-MS/MS	liquid chromatography with tandem mass spectrometry detection
ID	isotope dilution
IDMS	isotope dilution mass spectrometry
INTI	National Institute of Industrial Technology, Buenos Aires, Argentina
INRAP	National Institute of Research and Physical and Chemical Analysis, Tunisia
KC	Key Comparison
KCRV	Key Comparison Reference Value
KEBS	Kenya Bureau of Standards, NMI: Kenya
LC	liquid chromatography
MADe	median absolute deviation from the median (MAD)-based estimate of s: MADe = 1.4826·MAD, where MAD = median($\|x_i$-median(x_i)$\|$)
MRM	multiple reaction monitoring
NICOB	NIST Consensus Builder
NIMT	National Institute of Metrology of Thailand, Thailand
NMISA	National Measurement Institute South Africa, NMI: South Africa
NMI	national metrology institute
NMR	nuclear magnetic resonance spectroscopy
OAWG	Organic Analysis Working Group
pKow	logarithm of the octanol-water partition coefficient
PSE	pressurized solvent extraction
qNMR	quantitative nuclear magnetic resonance spectroscopy
QuEChERS	“Quick, Easy, Cheap, Effective, Rugged, Safe” liquid/solid extraction
RMP	Reference Measurement Procedure
SIM	selected ion monitoring
SPE	solid phase extraction
SRM	Selected reaction monitoring
UME	National Metrology Institute of Turkey, NMI: Turkey
VNIIM	D.I. Mendeleyev Institute for Metrology, DI: Russia

SYMBOLS

d_i	degree of equivalence: x_i - KCRV
$\%d_i$	percent relative degree of equivalence: $100 \cdot d_i$/KCRV
k	coverage factor: $U(x) = k \cdot u(x)$
n	number of quantity values in a series of quantity values
s	standard deviation of a series of quantity values: $s = \sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2/(n-1)}$
t_s	Student's *t*-distribution expansion factor
$u(x_i)$	standard uncertainty of quantity value x_i
$\bar{u}(x)$	pooled uncertainty: $\bar{u}(x) = \sqrt{\sum_{i=1}^{n} u^2(x_i)/n}$
$U(x)$	expanded uncertainty
$U_{95}(x)$	expanded uncertainty defined such that $x \pm U_{95}(x)$ is asserted to include the true value of the quantity with an approximate 95 % level of confidence
$U_{k=2}(x)$	expanded uncertainty defined as $U_{k=2}(x) = 2 \cdot u(x)$
x	a quantity value
x_i	the i^{th} member of a series of quantity values
$\bar{x}$	mean of a series of quantity values: $\bar{x} = \sum_{i=1}^{n} x_i/n$
z_i	z-score, a standardized quantity value: $z_i = (x_i - \bar{x})/s$

INTRODUCTION

Dried fig, which is known to be a healthy food with its high nutritional value, could either be consumed directly or can be made into a paste/slurry to be used in desserts and candies [5]. The agricultural practices in the production of dried fig such as ripening, harvesting and sun-drying, present significant risk of fungal infection and subsequent mycotoxin contamination. In many products, severe limitations have been introduced by the European Union (EU) (Commission Regulation No 1058/2012 amending Regulation No 1881/2006) and the maximum limits have been established in the European legislation for various mycotoxins, which are extremely toxic, carcinogenic, tetratogenic and hepatotoxic such as aflatoxins B_1, B_2, G_1 and G_2. Due to the high level of aflatoxins, some products exported to the EU have been rejected and withdrawn. Weekly alert notifications are released on the internet for the member states through a Rapid Alert System, which is considered very important to protect both consumers and producers prior to consuming/processing [6-11]. The major producers of dried fig are Turkey, USA, Iran and Mediterranean countries, among which Turkey, as the producer of 60 % of the total worldwide supply, is involved in half of the international trade in dried figs. This makes Turkey a major exporter of dried fig, which requires it to comply with the internationally accepted sanitation and hygiene standards during production, storage and delivery. Thus, presence of any aflatoxin contamination in exported figs needs to be monitored according to Commission Regulation (EU) No 1058/2012 of 12 November 2012 amending Regulation (EC) No 1881/2006 as regards maximum levels for aflatoxins in dried figs and measured through reliable and traceable methods, which require pure and matrix certified reference materials.

Extraction, chromatographic separation, and quantification of low-concentration organic compounds in complex matrices are core challenges for reference material producers and providers of calibration services. Evidence of successful participation in formal, relevant international comparisons are needed to document measurement capability claims (CMCs) made by national metrology institutes (NMIs) and designated institutes (DIs).

In April 2015, the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) approved the Key Comparison (KC) CCQM-K138 Determination of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs) in Dried Fig. CCQM-K138 was designed to assess participant capabilities for determination of mid-polarity contaminants in a food matrix. AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs can be successfully evaluated using either Liquid chromatography (LC) Mass spectrometry, high performance liquid chromatography (HPLC) with different detection methods. Aflatoxins must be removed by extraction, following cleanup.

The following sections of this report document the timeline of CCQM-K138, the measurands, study material, participants, results and the measurement capability claims that participation in CCQM-K138 can support. The Appendices reproduce the official communication materials and summaries of information about the results provided by the participants.

TIMELINE

Table 1 lists the timeline for CCQM-K138.

Table 1: Timeline for CCQM-K138

Date	Action
Apr 2015	Proposed to CCQM
October 2015	Draft protocol presented to OAWG as potential Track A or C Key Comparison
November 2015	OAWG authorized CCQM-K138 as a Track C Key Comparison; protocol approved
November 2015	Call for participation to OAWG members
March 2016 to June 2016	Study samples shipped to participants. The range in shipping times reflects delays from shipping and customs.
September 2016	Results due to coordinating laboratory
October 2016	Draft A report distributed to OAWG
Apr 2018	Draft B report distributed to OAWG
TBD	Final report approved by OAWG

MEASURANDS

The measurands to be determined are the mass fractions of Aflatoxin (B_1, B_2, G_1, G_2 and total) in dried fig. The structures of Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) are given in Figure 1. The nominal values of Aflatoxins B_1, B_2, G_1, G_2 and total Aflatoxin are between mass fractions of 3 - 7 ng/g, 0.3 - 1 ng/g, 1 - 3 ng/g, 0.08 - 0.3 ng/g and 6 - 9.5 ng/g, respectively.

Figures 1- 4 below display the molecular structure of B_1, B_2, G_1 and G_2.

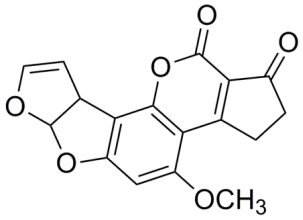

Figure 1: Structure of AFB_1

Aflatoxin B_1
AFB_1
pK_{OW} 1.23

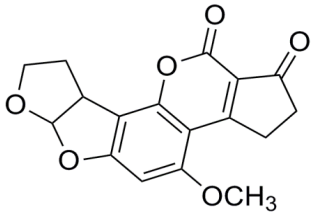

Figure 2: Structure of AFB_2

Aflatoxin B_2
AFB_2
pK_{OW} 1.45

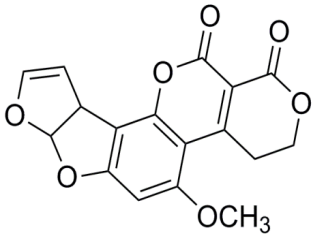

Figure 3: Structure of AFG_1

Aflatoxin G_1
AFG_1
pK_{OW} 0.50

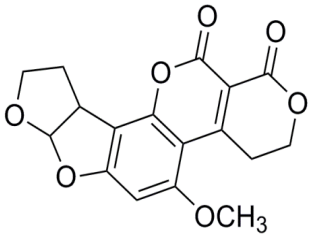

Figure 4: Structure of AFG_2

Aflatoxin G_2
AFG_2
pK_{OW} 0.71

STUDY MATERIALS

The test material is a candidate material for a dried fig certified reference material (CRM 1302).

Raw materials used in the production of dried figs were obtained from the Aydın province that meets about 70-75% of the production in Turkey. 300 kg of uncontaminated dried fig and 25 kg of dried fig contaminated by aflatoxin as Sarı Lop (Calimyrna) type were supplied from an exporting company in Aydın province as a starting material for the production of certified reference materials of aflatoxins in dried fig. The starting material was examined considering the visual UV findings before beginning of the process. All raw materials were subjected to gamma irradiation at around 5.3 kGy to prevent any microbiological activity. Since the aflatoxin content of the starting material was known to be stable under dry, dark and cold conditions, raw material was then kept in cold storage rooms at -18°C until the processing.

One of the most important and critical steps in the processing was the lyophilization, which is necessary to reduce moisture content of the material to minimize biological activity and improve long term storage stability. Lyophilization process was optimized for powder fig material which was obtained with the use of a blender homogenizer (Robot Coupe, Blixer 23, USA) with the

addition of ~13 % moisture-retaining material. The flowchart of the production process is given in Figure 5.

After lyophilization (loss of mass was ~14%) and blending processes, all powder material was sieved with 500 µm sieve. After homogenization with 3-D mixer (3-D MegaMix, HKTM, Turkey), material was bottled (as 160 g to each bottle) using a semi-automatic filling machine (Augapack, Vectofill, Belgium) and capped materials were subjected to second gamma irradiation (5.3 kGy) before storing at -80°C.

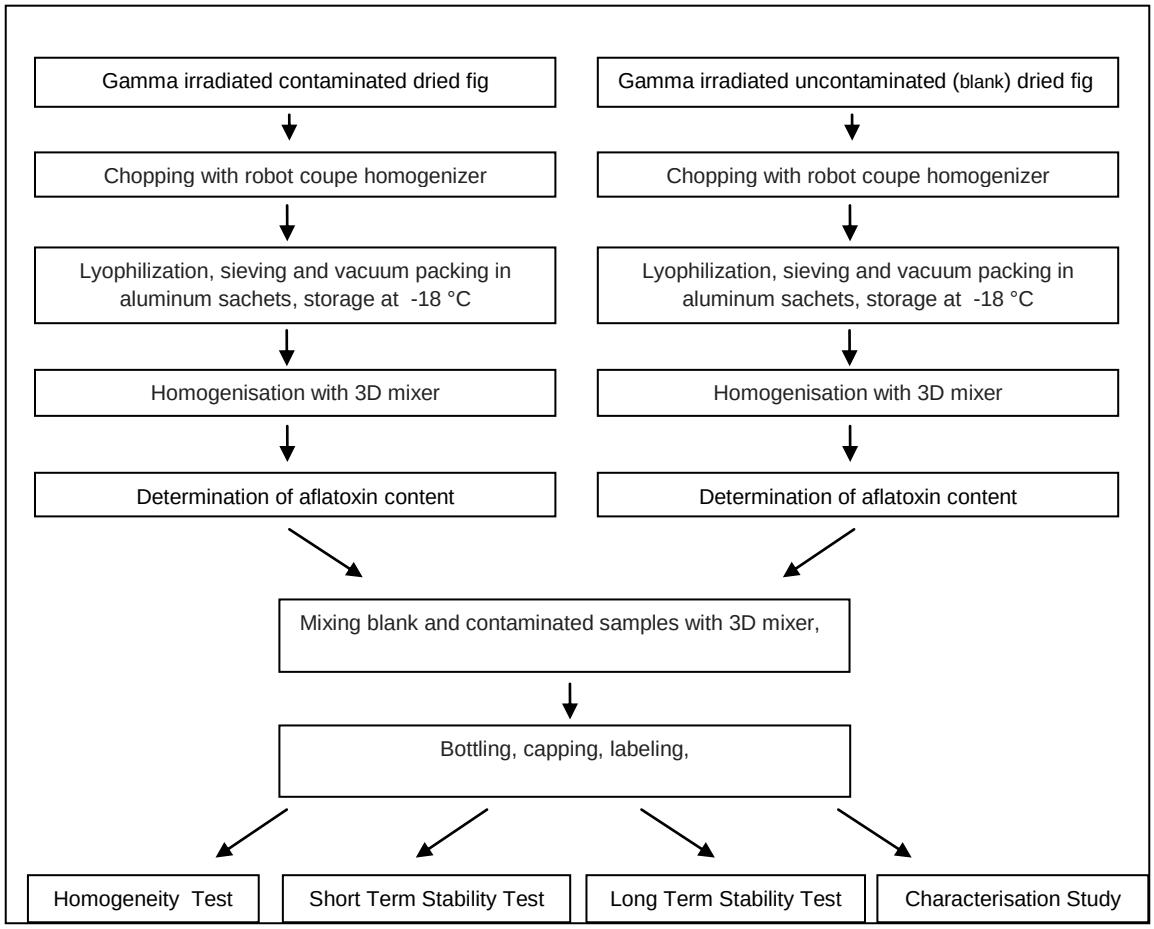

Figure 5: The flowchart of the production process of dried fig

The powder product obtained from the production was filled into light-impermeable airtight brown bottles as 160 g. Totally 511 units were produced. Samples were randomly selected with TRaNS and subjected to homogeneity, stability and characterisation tests. The results obtained by the analysis of selected units were evaluated statistically.

Each participant received 2 units of candidate reference material: HDPE bottles into aluminum sachet, containing about 160 g of powder dried fig. The recommended minimum sample amount for analysis was at least 6 g. Measurement results were to be reported on as received basis.

Homogeneity Assessment of Study Material

Homogeneity study between the units was performed to show that the assigned value was valid for all units within the stated uncertainty. In this study, 10 units were selected by using random stratified sampling software (TRaNS) and were reserved for the study of homogeneity between units. Homogeneity tests were carried out for all analytes of candidate CRM by measuring 3 sub-samples (6 g sample size) under repeatability conditions. The method used for these measurements was validated and the samples to be analysed were introduced to the instrument by random order to find out any trend arising from analytical and/or filling sequences. All homogeneity measurements were carried out using HPLC-FLD method.

The data for all analytes were evaluated statistically by regression analysis for the presence of any trend in analytical and filling sequence. After evaluation of data, no trend was found for any analyte in CRM candidate at 95% confidence level.

Grubbs test was applied to all data for the presence of outlier at 95% confidence level. According to data obtained for each analyte, it was found that the distribution was found to be normal and no outliers were found (Table 2).

Table 2: Statistical Evaluation Result of Homogeneity of the Study Material

Analyte	Is there aTrend?		Is there an Outlier?		Distribution
	Analytical sequence	Filling sequence	All data	Unit averages	All data
AFB_1	No	No	No	No	Normal/unimodal
AFB_2	No	No	No	No	Normal/unimodal
AFG_1	No	No	No	No	Normal/unimodal
AFG_2	No	No	No	No	Normal/unimodal
Total AF	No	No	No	No	Normal/unimodal

Analysis of Variance (ANOVA) is a statistical tool used to estimate the uncertainty contribution from homogeneity of the materials. All data were examined for normal data distribution using Shapiro-Wilk test and histograms before applying one way ANOVA test. All analytes (AFB_1, AFB_2, AFG_1, AFG_2 and total AF) showed normal distribution on Shapiro-Wilk test and histogram diagrams. The uncertainties of homogeneity between units were evaluated with one way ANOVA for all analytes. The equation (1) was used for the calculation of the repeatability of the method (s_{wb}) and equation (2) was used for the calculation of standard deviation between units (s_{bb}).

$$S_{wb} = \sqrt{MS_{within}} \quad \textbf{(1)}$$

where

MS_{within} : mean of square of variance within the unit

s_{wb} equals to "s" of the method as long as sub samples represent the whole unit.

$$S_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \quad \textbf{(2)}$$

where,

$MS_{between}$: mean of square of variance between units

n : number of replicates per unit

$MS_{between}$ is found to be smaller than MS_{within} in conditions for which the heterogeneity of the material was smaller than heterogeneity that can be determined by the applied analytical method or measurement fluctuations that may have occurred randomly. In these cases, since s_{bb} cannot be calculated, u^*_{bb} was calculated as heterogeneity contributing to uncertainty including method repeatability using equation (3).

$$u^*_{bb} = \frac{S_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{V_{MSwithin}}} \quad \textbf{(3)}$$

where,

$v_{MSwithin}$: degree of freedom of MS_{within}

The uncertainty values obtained from the homogeneity study are given in Table 3.

Table 3: Homogeneity Results of the Study Material

Analyte	Average value (ng/g)	$s_{bb,rel}$	$u^*_{bb,rel}$	$u_{bb,rel}$
AFB_1	5.38	2.27	2.28	2.28
AFB_2	0.60	$MS_{between}<MS_{within}$	4.07	4.07

AFG_1	2.21	7.46	4.31	7.46
AFG_2	0.18	4.00	4.68	4.68
Total AF	8.37	1.52	2.05	2.05

The values of $MS_{between}$ were found to be smaller than the values of MS_{within} for analyte AFB_2. So, $\mathbf{u^*_{bb}}$ was calculated and used as the uncertainty contribution due to homogeneity. For the cases where both s_{bb} and u^*_{bb} can be calculated, the bigger one was taken as uncertainty contribution due to between bottle homogeneity (u_{bb}).

The Results of the homogeneity assessment for AFB_1, AFB_2, AFG_1, AFG_2 in dried fig are given at Table 4.

Table 4: Results of the homogeneity assessment for AFB_1, AFB_2, AFG_1, AFG_2 in dried fig.

ANOVA Estimate	AFB_1	AFB_2	AFG_1	AFG_2	Total AFs
Within-packet, CV_{wth}:	7.34%	13.1%	13.9%	15.1%	6.60%
Between-packet, CV_{btw}:	2.27%	$MS_{between}<MS_{within}$	7.46%	4.00%	1.52%
Total analytical variability, CV:	2.28%	4.07%	4.31%	4.68%	2.05%
Probability of falsely rejecting the hypothesis that all samples have the same concentration:	76%	39%	83%	65%	56%

Stability Assessment of Study Material

Stability studies were performed with an isochronous design which is cited in ISO Guide 35. For the Short Term Stability (STS) test, two different temperatures (-20˚C and 4˚C) and 4 time points (1, 2, 3 and 4 weeks) were tested. 10 samples were selected by TRaNS. 8 samples were subjected to the test temperatures for the specified time intervals.

Samples were moved to -80˚C (reference temperature) after completion of the test time. All samples were analysed at the same time. Two replicate samples were prepared from each unit (6 g sample size) and were analyzed by HPLC-FLD method under the repeatability conditions for determining the mass fractions of AFB_1, AFB_2, AFG_1, AFG_2 and total AF.

The data for each temperature were first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. The number of detected outliers is given in the Table 5.

Since no technical reason can be found to reject these data, all outliers were included in the STS calculations.

Values calculated for each time point were plotted against the time for the assessment of short term stability. The relationship between variables were analyzed in order to determine if any significant change exists in mass fraction values with the testing time (*regression analysis*). It was found that the slopes were not significantly different than zero for all in the 95% confidence interval.

Uncertainty calculations were done using equation (4). The maximum time for transfer was chosen as 2 weeks.

$$u_{STS} = \frac{RSD}{\sqrt{\sum(t_i - \bar{t})^2}} x\ t \qquad (4)$$

where,

RSD : relative standard deviation obtained from all data in STS

t_i : time point for each replicate

$\bar{t}$: mean of all time points

t : maximum time suggested for transfer: 2 weeks

Results obtained from short term stability are given in Table 5.

Table 5: Short Term Stability Test Results

Analyte	-20 °C $u_{sts,rel}$ (%) for 2 weeks	4 °C $u_{sts,rel}$ (%) for 2 weeks	Number of outliers in 95% confidence interval*		Number of outliers in 99% confidence interval*		Is there a significant trend in 95% confidence interval?		Is there a significant trend in 99% confidence interval?	
			-20 °C	4 °C	-20 °C	4 °C	-20 °C	4 °C	-20 °C	4 °C
AFB_1	2.6	2.9	1	-	1	-	No	No	No	No
AFB_2	2.1	2.8	-	-	-	-	No	No	No	No
AFG_1	6.5	4.9	-	-	-	-	No	No	No	No
AFG_2	6.1	4.6	-	-	-	-	No	No	No	No
Total AF	3.3	4.0	1	-	-	-	No	No	No	No

* One-sided Grubbs Test

Result of this study showed that the sample could be transferred to the end users within a two week time interval ensuring the temperature not to exceed + 4°C with cooling elements.

Stability Assessment of Study Material (Long Term)

Shelf life of the produced CRM was determined by the long-term stability (LTS) studies. +4 ^{o}C was chosen as the test temperature for long term stability tests and in total 10 units were reserved for this study. Samples were selected by TRaNS software and kept at +4 ^{o}C for 9 months. Two units for each time point (0, 2, 4, 6, and 9 months) were stored at +4 ^{o}C and transferred to -80˚C (reference temperature) after completion of the test time. Two replicate samples (6 g sample size) were prepared from each unit and analyzed by HPLC-FLD under the repeatability conditions for determining the mass fractions of AFB_1, AFB_2, AFG_1, AFG_2 and total AF.

The data was first examined by one-sided Grubbs test for both 95% and 99% confidence intervals to find out outliers. The numbers of detected outliers are given in the Table 5. Since no technical reason was present to reject these data, all outliers were included in the LTS calculations.

Values calculated for each time point were plotted against the time for the assessment of LTS. The relationship between variables were analyzed in order to determine if any significant change exists in mass fraction values with the testing time (regression analysis). It was found that the slopes were not significantly different than zero for all analytes in the 95% confidence interval. The potential uncertainty contribution of long term stability, u_{lts}, was calculated using equation (5) for 1 year of shelf life at +4 ^{o}C.

$$u_{LTS} = \frac{RSD}{\sqrt{\sum(t_i - \bar{t})^2}} x\ t \qquad (5)$$

where,

RSD : relative standard deviation obtained from all data in LTS

t_i : time point for each replicate

$\bar{t}$: mean of all time points

t : shelf life suggested at +4 ^{o}C: 1 year

Table 6: Long Term Stability Test Results of the Study Material

Analyte	$u_{lts,rel}$ (%) at +4 °C for 1 year	Number of outliers in 95% confidence interval*	Number of outliers in 99% confidence interval*	Is there a significant trend in 95 % confidence interval?	Is there a significant trend in 99% confidence interval?
AFB_1	11.1	-	-	No	No
AFB_2	13.5	1	-	No	No
AFG_1	12.8	1	1	No	No
AFG_2	13.5	-	-	No	No
Total AF	10.1	-	-	No	No

* Single Grubbs Test

PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION

The call for participation was distributed in November 2015 with the intent to distribute samples in February 2016, receive results in July 2016, and discuss results at the CCQM OAWG meeting, October 2016. See Table 1 for study timeline. Appendix A reproduces the Call for Participation; Appendix B reproduces the study Protocol.

Table 7 lists the institutions that registered for CCQM-K138

Table 7: Institutions Registered for CCQM-K138

NMI or DI	Code	Country	Contact
Bundesanstalt fuer Materialforschung und –pruefung	BAM	Germany	Matthias Koch Matthias.Koch@bam.de
Chemical Metology Laboratory (General Chemical State Laboratory - Hellenic Metrology Institute)	EXHM/GCSL-EIM	Greece	Elias Kakoulides metrology@gcsl.gr
Government Laboratory, Hong Kong	GLHK	Hong Kong	Andy Chan cmchan@govtlab.gov.hk
National Institute of Industrial Technology, Toxicology and Nutrition Laboratory	INTI	Argentina	Estela Kneeteman estelak@inti.gob.ar
Kenya Bureau of Standards, Food and Agriculture	KEBS	Kenya	Mr. Isaac Mugenya mugenya@kebs.org
National Institute of Metrology of Thailand	NIMT	Thailand	Cheerapa Boonyakong cheerapa@nimt.or.th
National Metrology Institute of South Africa	NMISA	South Africa	Maria Fernandes-Whaley MFWhaley@nmisa.org

D.I. Mendeleyev Institute for Metrology	VNIIM	Russia	Anatoliy Krylov ak@vniimex.ru
TUBITAK UME, National Metrology Institute	TUBITAK UME	Turkey	Ahmet Ceyhan Gören Taner Gokcen

The participants were informed of the date of dispatching of samples. Each participant received 2 units of candidate reference material (HDPE bottles into aluminium sachet containing about 165 g of powder dried fig).

Due to delays in sample shipping and customs issues, the last set of material was delivered in June 2016. Because of these delays, the deadline for submission of results was postponed to 30 Sep 2016.

The participants were requested to report results from the mean of two samples, with corresponding standard and expanded uncertainty. The value of the results and their associated standard uncertainties must be expressed in ng/g. If the final result has been calculated from more than one method, the individual results from the contributing methods must also be reported. Participants were asked to provide information about the applied analytical procedure including the sample preparation and calibration methods and their metrological traceability. Each participant was asked to make an assessment of the measurement uncertainty. Each variable contributing to the uncertainty of the result was to be identified and quantified in order to be included in the combined standard uncertainty of the results. A full uncertainty budget was to be reported, as part of the results. All cells in all sheets (Result Reporting Form, Method Information, Comparison Results and Moisture Content Method) in Annex 2 “Report Form” was requested to be filled out in the Excel file provided in electronic form by TUBITAK UME.

RESULTS

Participants were requested to report a single estimate of the mass fraction ng/g for AFB_1, AFB_2, AFG_1, AFG_2 and total AF of independent measurements of two bottles. Results ranged from 5.17 to 7.27 ng/g with an %RSD of 10.47 for AFB_1, ranged from 0.60 to 0.871 ng/g with an %RSD of 11.69 for AFB_2, ranged from 1.98 to 2.6 ng/g with an %RSD of 10.36 for AFG_1, ranged from 0.06 to 0.32 ng/g with an %RSD of 35.6 for AFG_2, and ranged from 8.29 to 10.31 ng/g with an %RSD of 7.69 for Total AF.

In addition to the quantitative results, participants were instructed to describe their analytical methods, approach to uncertainty estimation, and the Core Competencies they felt were demonstrated in this study. Appendices C, D, and E reproduce the relevant report forms.

CCQM-K138 results were received from 9 of the 9 institutions that received samples.

Calibration Materials Used by Participants

Participants used a range of different calibration materials, in several cases from commercial providers. Table 8 lists the calibrants used by each institute and how participants attempted to establish the traceability of the calibrants, where this was carried out. If this was via their own measurements, its assigned purity, the method used, and how the participant had demonstrated their competence in the use of the method(s) were also given in Table 8.

The issue of calibrant traceability was discussed at the OAWG meeting in September 2017. At that meeting it was flagged that many of the calibration materials employed did not meet the CIPM traceability requirements from CIPM 2009-24. This document allows two pathways: in house assessment using capabilities whose effectiveness has been demonstrated or the use of another NMI/DI's capabilities where they have also been demonstrated.

The commercial materials used did not meet these CIPM criteria and thus where institutes did not carry out an independent in-house assessment then results using these calibrants could not be included in the KCRV. One instance that caused particular issue was the use of the IRMM ERMs. Several institutes used these materials assuming they would meet the CIPM traceability requirements, however these are certified by consensus from a range of different laboratories and hence they were not deemed to be acceptable.

Two institutes carried out in house assessment of the commercial calibrants in a way that was deemed sufficient to provide traceability.

EXHM purity assigned pure materials by mass balance and qNMR and then made up gravimetric solutions and measured them via IDMS versus the IRMM solutions. The purities of the AFs were given as below:

AFB_1=96.13 ± 3.18%, AFB_2=93.32 ± 3.13%, AFG_1=98.60 ± 3.35%, AFG_2=94.02 ± 3.12% (k=3 due to limited material)

The values on the certificate of the IRMM-ERM materials were:

AFB_1=3.79 µg/g ± 2.90 %, AFB_2=3.80 µg/g ± 2.11%, AFG_1=3.78 µg/g ± 3.44%, AFG_2=3.80 µg/g ± 1.84%, (k=2). The determined values by EXHM agreed with these values within their uncertainties.

TUBITAK UME purity assigned commercially available highly-pure substances by in-house qNMR purity assignment traceable to UME CRM 130. The purities of the AFs were given as below:

AFB_1=85.47 ± 0.94%, AFB_2=83.35 ± 1.25%, AFG_1=77.13 ± 4.77%, AFG_2=70.18 ± 0.46%.

The values of the Sigma standards were:

AFB_1=99.64% AFB_2=98.50%, AFG_1=100%, AFG_2=100%.The values used by UME for these materials were those assigned in-house.

Some other institutes did carry out assessment of materials. BAM used checks versus different lot numbers and different suppliers but as they were all commercial materials this was not deemed sufficient, BAM did use LC-MS for identity confirmation. INTI and KEBS used spectrophotometric analysis of their commercial calibration solutions however this was also deemed inappropriate. NIMT, NMISA and GLHK used the IRMM calibrants with no assessment and VNIIM used the Biopure materials with no assessment.

As a result of the full analysis of the approaches used by all participants, due to the traceability requirements for the calibrants not being met, the results of KEBS, INTI, VNIIM and BAM were excluded from KCRV determination. If the institutes that had employed the IRMM materials were also excluded this would have left two institutes valid for the KCRV calculation. In this case a compromise was agreed to whereby it was deemed that the work done by EXHM had demonstrated the IRMM materials had valid assigned values and in this case the institutes that utilised those materials would have their results included.

It is noted that all institutes except EXHM and UME would need to use different approaches to their calibration if they wished to have a CMC considered associated with this comparison considered.

Table 8: Metrological Traceability of Participants' Results

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	Purity Techniques[b]	Evidence of Competence
BAM	AFB_1 AFB_2 AFG_1 AFG_2	Gravimetric sample preparation Aflatoxin B_1: 16192B Aflatoxin B_2: 15483A Aflatoxin G_1: 15331C Aflatoxin G_2: 15391A	Biopure	-	Certified standard solutions used. Purities of calibration standards were independently confirmed by LC-MS measurements (scan mode; ESI+/-). The specified aflatoxin contents of the used certified standard solutions were cross checked by certified standards of different lot numbers (same provider) and certified standard solutions of a second provider	N/A
EXHM / GCSL-EIM	AFB_1 AFB_2 AFG_1 AFG_2	IRMM-ERM-AC057 IRMM-ERM-AC058 IRMM-ERM-AC059 IRMM-ERM-AC060	IRMM	Commercial solid aflatoxins: AFB_1=96.13±3.18%, AFB_2=93.32±3.13%, AFG_1=98.60±3.35%, AFG_2=94.02±3.12%.	The solid aflatoxins were characterized for their purity using the Mass Balance approach and qNMR. The concentration of the solutions prepared gravimetrically were assigned against the IRMM CRM solutions (ERM AC 057, 058, 059, 060) using IDMS experiments The values assigned were found to agree with the gravimetric preparations within the stated uncertainties	l Participation in CCQM-K104, P117.c, CCQM-K131, CCQM-K78 underpins claimed uncertainties

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	Purity Techniques[b]	Evidence of Competence
GLHK	AFB_1 AFB_2 AFG_1 AFG_2	IRMM-ERM-AC057 IRMM-ERM-AC058 IRMM-ERM-AC059 IRMM-ERM-AC060	IRMM	N/A		
INTI	AFB_1 AFB_2 AFG_1 AFG_2	Aflatoxin B_1 Cat. Code: 5032 Aflatoxin B_2 Cat. Code: 5033 Aflatoxin G_1 Cat. Code:5035 Aflatoxin G_2 Cat. Code: 5036	Fluka AG	Manufacturer declaration using TLC and HPLC (more than 98%)	One solution of each aflatoxin was prepared to obtain 4 stock solutions of 8-10 ug/ml in acetonitrile. These solutions were verified using an Spectrophotometric method (AOAC 971.22). After the measurement of the stock solution at 350nm, it was adjusted the purity of each calibration solution. The assignment of purity was determined following the next equation: % purity = $\frac{cc_{standard\ stock} \times 10ml \times 5000ul \times 100}{50\ ul \times 1000\ ug/mg \times 10\ mg}$	N/A

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	Purity Techniques[b]	Evidence of Competence
KEBS	AFB_1 AFB_2 AFG_1 AFG_2	AFB_1 Lot# AF017 AFB_2 Lot# 141104-070 AFG_1 Lot# 150305-070 AFG_2 Lot# 150309-070	Fermentek Trilogy Analytical Trilogy Analytical Trilogy Analytical	-	HPLC/FLD	N/A
NIMT	AFB_1 AFB_2 AFG_1 AFG_2	IRMM-ERM-AC057 IRMM-ERM-AC058 IRMM-ERM-AC059 IRMM-ERM-AC060	IRMM	N/A		
NMISA	AFB_1 AFB_2 AFG_1 AFG_2	IRMM-ERM-AC057 IRMM-ERM-AC058 IRMM-ERM-AC059 IRMM-ERM-AC060	IRMM	N/A		

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	Purity Techniques[b]	Evidence of Competence
VNIIM	AFB_1 AFB_2 AFG_1 AFG_2	Aflatoxin B_1 in acetonitrile Aflatoxin B_2 in acetonitrile Aflatoxin G_1 in acetonitrile Aflatoxin G_2 in acetonitrile	Biopure	N/A		
TUBITAK UME	AFB_1 AFB_2 AFG_1 AFG_2	AFB_1 A6636 AFB_2 A9887 AFG_1 A0138 AFG_2 A0263	Sigma	AFB_1=85.471 ± 0.943%, AFB_2=83.351 ± 1.253%, AFG_1=77.131 ± 4.767%, AFG_2=70.178 ± 0.455%	Purity of commercially available highly-pure substances were determined by in-house qNMR purity assignment traceable to UME CRM 1301	Participation in CCQM-K55b-d underpins claimed uncertainties

a Stated as Value ± U_{95}(Value)

b DSC: Differential scanning calorimetry
GC-FID: Gas chromatography with flame ionization detection
HPLC-DAD: High pressure liquid chromatograph with diode-array detection
MB: Mass balance
qNMR: Quantitative nuclear magnetic resonance

Methods Used by Participants

Each laboratory was requested to use a properly validated method, calibration standards with a metrologically traceable assigned value (an appropriate CRM or material where its purity has been suitably assessed by the participant) according to criteria established by the CCQM OAWG for the inclusion of results in the calculation of the KCRV.

All participants based their analyses on LC-MS/MS, HR-LC-MS and HPLC-FLD. Brief descriptions of the analytical methods used by the participants, including sample preparation, analytical technique, calibrants and quantification approach is summarized in Appendix F Tables F1-5. The participants' approaches to estimating uncertainty are provided in Appendix G.

The spread of results for each analyte was reasonably broad but there was no trend observed from the techniques used. INTI and KEBS used fluorescence detection whereas all other participants used IDMS. Significant effort was put into sample clean up by most participants, with immunoaffinity clean up being the most common. Only BAM used a simple centrifugation step which may have provided less selectivity.

Participant Results for AFB_1, AFB_2, AFG_1, AFG_2 and total AFs

The results for CCQM-K138 for the determination of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) are detailed in Table 9 - 13 and presented graphically in Figure 6 -10 respectively. Results are ranging from 5.17 to 7.27 ng/g with an %RSD of 10.47 for AFB_1, ranging from 0.60 to 0.871 ng/g with an %RSD of 11.69 for AFB_2, ranging from 1.98 to 2.6 ng/g with an %RSD of 10.36 for AFG_1, ranging from 0.06 to 0.32 ng/g with an %RSD of 35.64 for AFG_2, and ranging from 8.29 to 10.31 ng/g with an %RSD of 7.69 for Total AF.

Table 9: Reported Results for AFB_1, ng/g

	AFB_1, ng/g					
NMI	x	$u(x)$	$u(x)$ %	k	$U(x)$	$U(x)$ %
BAM	5.41	0.15	2.77	2.571	0.40	9.06
EXHM/GCSL-EIM	5.994	0.123	2.05	2.03	0.249	4.15
GLHK	5.8	0.5	8.62	2	1.1	18.97
INTI	5.17	0.33	6.38	2	0.66	12.77
KEBS	7.27	0.8	11.00	2	1.6	22.83
NIMT	6.6	0.40	6.06	2.03	0.9	13.64
NMISA	6.20	0.28	4.52	2	0.56	9.03
VNIIM	6.22	0.23	3.70	2	0.46	7.40
TUBITAK UME	5.72	0.33	5.77	2	0.66	11.54
n	9.00					
$\bar{x}$	6.04					
s	0.63					
CV	10.47					

n = number of results included in summary statistics; $\bar{x}$ = mean; s = standard deviation;
CV = $100 \cdot s/\bar{x}$

Table 10: Reported Results for AFB_2, ng/g

	AFB_2, ng/g					
NMI	*x*	*u*(*x*)	*u*(*x*) %	*k*	*U*(*x*)	*U*(*x*) %
BAM	0.66	0.03	4.55	2.571	0.08	12.12
EXHM/GCSL-EIM	0.871	0.022	2.53	2.11	0.047	5.40
GLHK	0.74	0.07	9.46	2	0.14	18.92
INTI	0.69	0.13	18.84	2	0.26	37.68
KEBS	0.6	0.1	16.67	2	0.2	33.33
NIMT	0.8	0.05	6.25	2.04	0.1	12.50
NMISA	0.755	0.04	5.30	2	0.08	10.60
VNIIM	0.81	0.06	7.41	2	0.12	14.81
TUBITAK UME	0.67	0.05	7.46	2	0.09	13.43
n	9.00					
$\bar{x}$	0.73					
s	0.09					
CV	11.69					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = $100 \cdot s/\bar{x}$

Table 11: Reported Results for AFG_1, ng/g

	AFG_1, ng/g					
NMI	*x*	*u*(*x*)	*u*(*x*) %	*k*	*U*(*x*)	*U*(*x*) %
BAM	2.01	0.11	5.47	2.571	0.27	13.43
EXHM/GCSL-EIM	2.093	0.061	2.91	2.07	0.125	5.97
GLHK	2	0.2	10.00	2	0.5	25.00
INTI	2.5	0.07	2.80	2	0.14	5.60
KEBS	2.39	0.4	16.74	2	0.8	33.47
NIMT	2.6	0.18	6.92	2.1	0.4	15.38
NMISA	2.24	0.2	8.93	2	0.4	17.86
VNIIM	1.98	0.11	5.56	2	0.22	11.11
TUBITAK UME	2.16	0.15	6.94	2	0.3	13.89
n	9.00					
$\bar{x}$	2.22					
s	0.23					
CV	10.36					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = $100 \cdot s/\bar{x}$

Table 12: Reported Results for AFG_2, ng/g

	AFG_2, ng/g					
NMI	*x*	*u*(*x*)	*u*(*x*) %	*k*	*U*(*x*)	*U*(*x*) %
BAM	0.22	0.01	4.55	2.571	0.03	13.64
EXHM/GCSL-EIM	0.264	0.01	3.79	2.2	0.022	8.33
GLHK	0.22	0.04	18.18	2	0.07	31.82
INTI	0.32	0.04	12.50	2	0.08	25.00
KEBS	0.06	0.01	16.67	2	0.02	33.33
NIMT	0.3	0.03	10.00	2	0.1	33.33
NMISA	0.214	0.025	11.68	2	0.049	22.90
VNIIM	0.15	0.04	26.67	2	0.08	53.33
TUBITAK UME	0.23	0.02	8.70	2	0.04	17.39
n	9.00					
$\bar{x}$	0.22					
s	0.08					
CV	35.60					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = $100 \cdot s/\bar{x}$

Table 13: Reported Results for Total AF, ng/g

	Total AFs ng/g					
NMI	*x*	*u*(*x*)	*u*(*x*) %	*k*	*U*(*x*)	*U*(*x*) %
BAM	8.29	0.19	2.29	2.571	0.49	5.91
EXHM/GCSL-EIM	9.223	0.141	1.53	2	0.282	3.06
GLHK	8.7	0.6	6.90	2	1.2	13.79
INTI	8.68	0.57	6.57	2	1.14	13.13
KEBS	10.31	1.34	13.00	2	2.68	25.99
NIMT	10.3	0.44	4.27	2.57	1.2	11.65
NMISA	9.4	0.65	6.91	2	1.3	13.83
VNIIM	9.16	0.27	2.95	2	0.54	5.90
TUBITAK UME	8.78	0.35	3.99	2	0.7	7.97
n	9.00					
$\bar{x}$	9.20					
s	0.71					
CV	7.69					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = $100 \cdot s/\bar{x}$

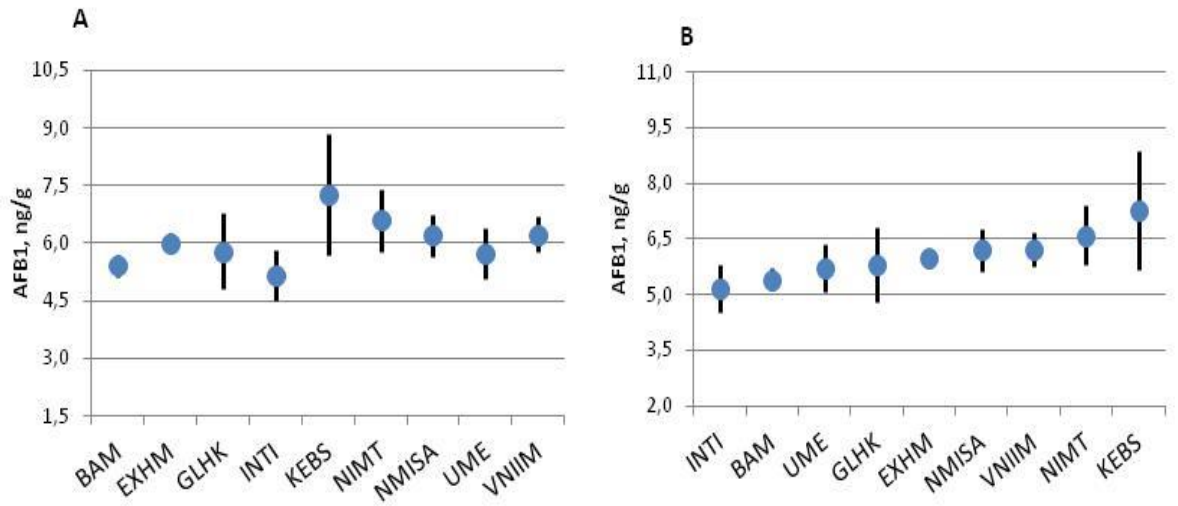

Figure 6: Reported Results for AFB_1, ng/g

Panels A and B display the reported results for AFB_1; panel A displays the results sorted alphabetically by NMI Acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their 95 % expanded uncertainties, *U*(*x*). The thin horizontal gridlines are provided for visual guidance.

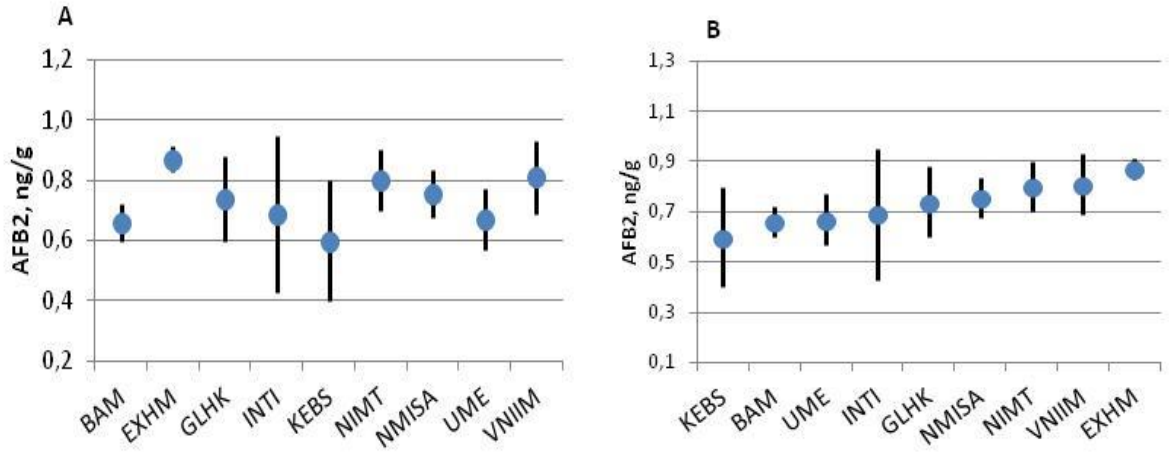

Figure 7: Reported Results for AFB_2, ng/g

Panels A and B display the reported results for AFB_2; panel A displays the results sorted alphabetically by NMI Acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their 95 % expanded uncertainties, *U*(*x*). The thin horizontal gridlines are provided for visual guidance.

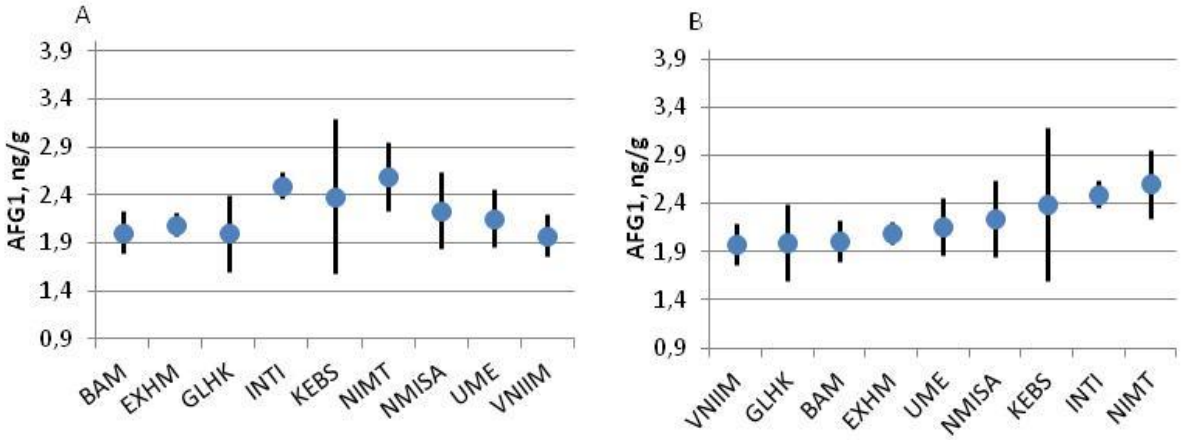

Figure 8: Reported Results for AFG_1, ng/g

Panels A and B display the reported results for AFG_1; panel A displays the results sorted alphabetically by NMI Acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their 95 % expanded uncertainties, *U*(*x*). The thin horizontal gridlines are provided for visual guidance.

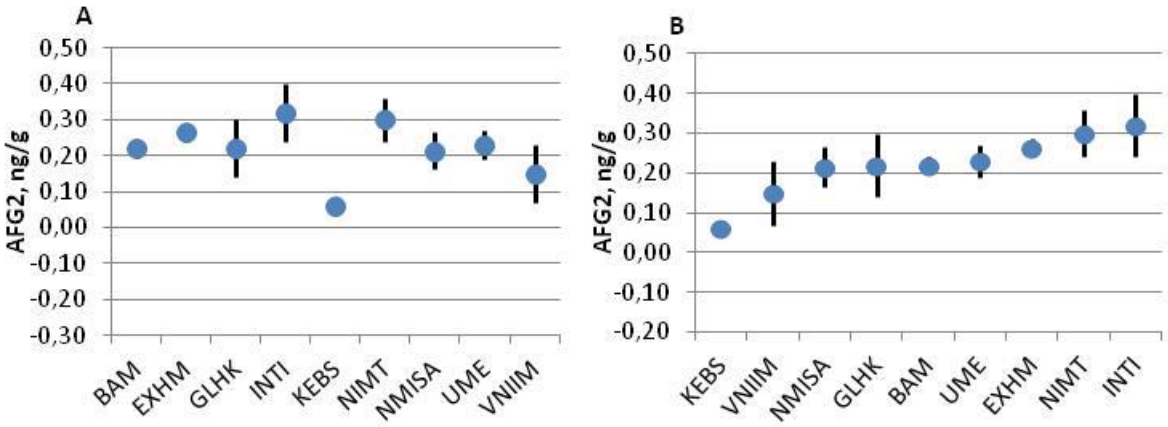

Figure 9: Reported Results for AFG_2, ng/g

Panels A and B display the reported results for AFG_2; panel A displays the results sorted alphabetically by NMI Acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their 95 % expanded uncertainties, *U*(*x*). The thin horizontal gridlines are provided for visual guidance.

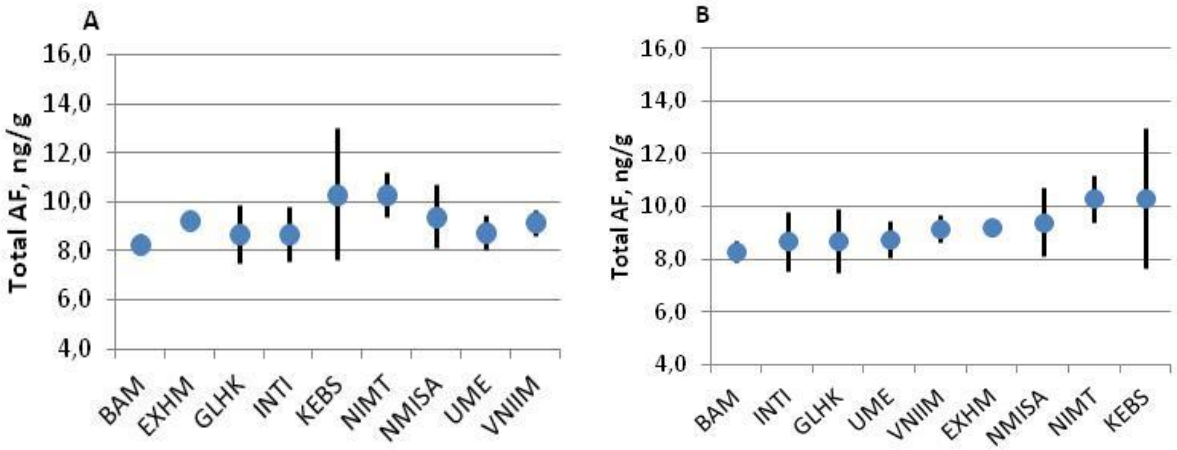

Figure 10: Reported Results for Total AF, ng/g

Panels A and B display the reported results for Total AF; panel A displays the results sorted alphabetically by NMI Acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, x; bars their 95 % expanded uncertainties, $U(x)$. The thin horizontal gridlines are provided for visual guidance.

Discussion of Results

The Draft A Report was sent to the participants to review in March 2017. The examination of the data revealed that NMISA correctly reported two individual results for AFG_2 however the mean was incorrectly calculated, they reported a corrected mean result for AFG_2 before the April 2017 OAWG meeting. The NMISA results for AFG_2 are given in table 14.

Table 14: Reported Results for AFG_2, ng/g

Participating Institutes	Overall Mean (ng/g)	u (ng/g)	k	U (ng/g)
NMISA (First result)	0.214	0.025	2	0.049
NMISA (Corrected result)	0.229	0.026	2	0.053

VNIIM had followed-up on their results following the October 2016 OAWG meeting, and in the April 2017 meeting they confirmed that their AFG_2 result remained unchanged at 0.15 ng/g.

KEY COMPARISON REFERENCE VALUE (KCRV)

Selecting an appropriate KCRV estimator for these small and reasonably variable datasets was carefully considered. It was decided at the OAWG meeting in September 2017 in Ottawa, for UME to consider the suitability of using a Linear Pool as a potential KCRV estimator. The linear pool estimator is suitable as it reflects the overall diversity amongst the individual results and calculates the KCRV as the average expected value that would be reported by any participant. It is considered a good estimator where there are small datasets with variability.

The results of Linear pool KCRV estimator are given in Table 15, in conjunction with other estimators that were considered. The Linear pool KCRV relative to the reported results for AFB_1, AFB_2, AFG_1, AFG_2 are presented graphically in Figure 11 -15.

Table 15. Candidate Key Comparison Reference Values

		AFB_1, ng/g			AFB_2, ng/g		
Estimator	u?[a]	X	$u(X)$	$U_{95}(X)$[b]	X	$u(X)$	$U_{95}(X)$[b]
Median	No	5.99	0.17	0.34	0.76	0.037	0.075
DL-Mean 1	No	6.022	0.066	0.183	0.774	0.037	0.103
DL-Mean 2	No	6.02	0.09	0.26	0.774	0.035	0.096
Bayesian	No	6.04	0.16	-0.30/+0.34	0.777	0.044	-0.092/+0.084
Linear Pool	Yes	6.06	0.47	-0.93/+1.01	0.766	0.083	-0.126/+0.134

		AFG_1, ng/g			AFG_2, ng/g		
Estimator	u?[a]	X	$u(X)$	$U_{95}(X)$[b]	X	$u(X)$	$U_{95}(X)$[b]
Median	No	2.16	0.066	0.133	0.23	0.013	0.027
DL-Mean 1	No	2.195	0.090	0.251	0.248	0.014	0.038
DL-Mean 2	No	2.20	0.095	-0.27/+0.26	0.248	0.015	0.040
Bayesian	No	2.18	0.098	-0.18/+0.21	0.250	0.016	-0.034/+0.029
Linear Pool	Yes	2.22	0.265	-0.46/+0.59	0.246	0.042	-0.079/+0.089

		Total AF, ng/g		
Estimator	u?[a]	X	$u(X)$	$U_{95}(X)$[b]
Median	No	9.22	0.367	0.735
DL-Mean 1	No	9.272	0.252	0.699
DL-Mean 2	No	9.27	0.27	-0.74/+0.73
Bayesian	No	9.27	0.33	-0.64/+0.68
Linear Pool	Yes	9.28	0.742	-1.35/+1.52

a) Does the estimator utilize the information in the reported uncertainties?
b) $U_{95}(X) = t_s \cdot u(X)$, where t_s is the appropriate two-tailed Student's t critical value for 95 % coverage.

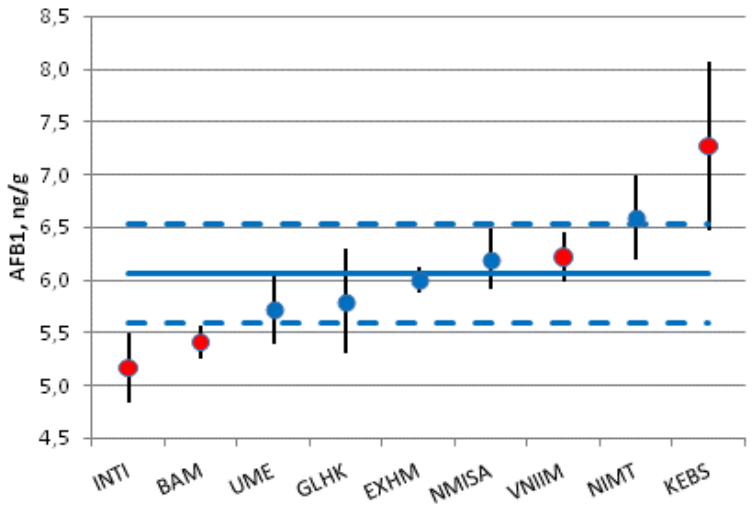

Figure 11: Linear pool KCRV relative to the reported results for AFB_1, ng/g

The results are sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their standard uncertainties, *u*(*x*). The blue horizontal line denotes the candidate KCRV. The bracketing dashed lines denote the standard uncertainty of the candidate KCRV. The red data points were not included in the KCRV calculation.

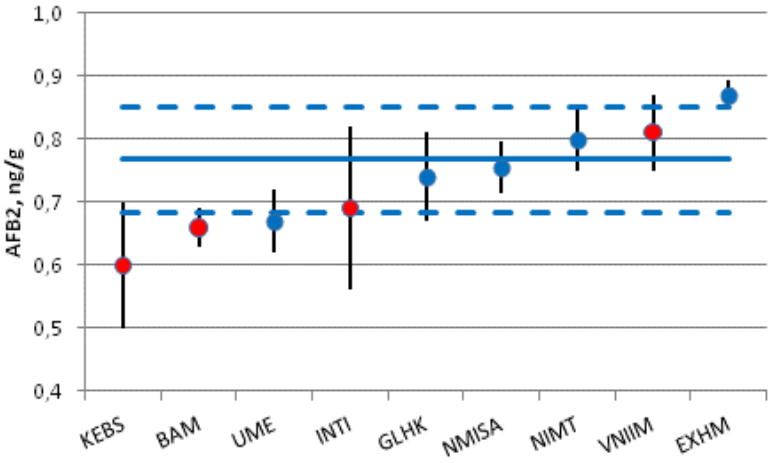

Figure 12: Linear pool KCRV relative to the reported results for AFB_2, ng/g

The results are sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their standard uncertainties, *u*(*x*). The blue horizontal line denotes the candidate KCRV. The bracketing dashed lines denote the standard uncertainty of the candidate KCRV. The red data points were not included in the KCRV calculation.

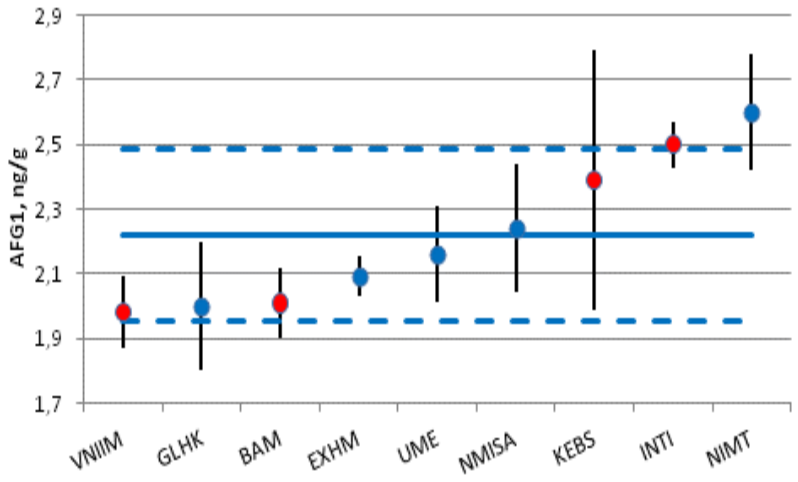

Figure 13: Linear pool KCRV relative to the reported results for AFG_1, ng/g

The results are sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their standard uncertainties, *u*(*x*). The blue horizontal line denotes the candidate KCRV. The bracketingdashed lines denote the standard uncertainty of the candidate KCRV. The red data points were not included in the KCRV calculation.

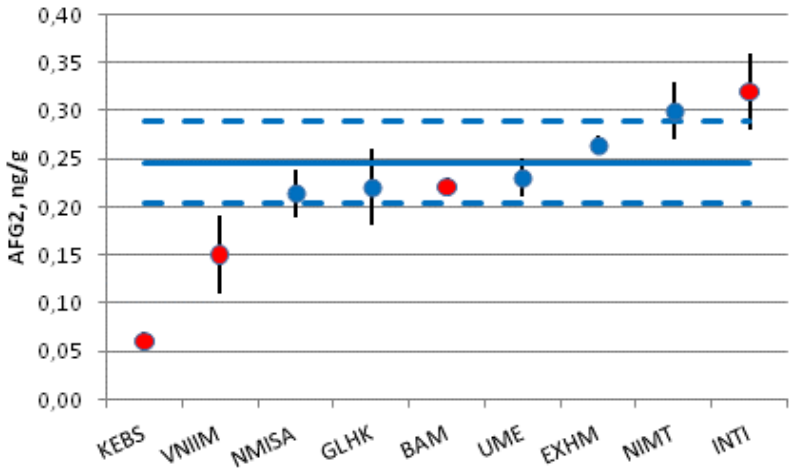

Figure 14: Linear pool KCRV relative to the reported results for AFG_2, ng/g

The results are sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their standard uncertainties, *u*(*x*). The blue horizontal line denotes the candidate KCRV. The bracketing dashed lines denote the standard uncertainty of the candidate KCRV. The red data points were not included in the KCRV calculation.

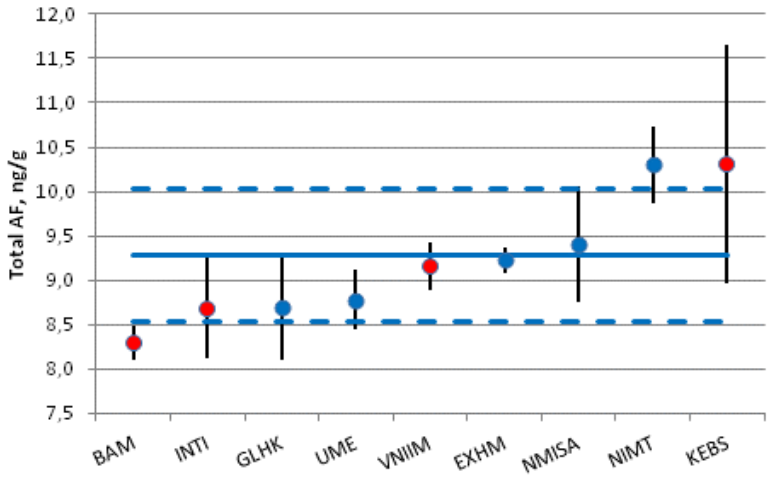

Figure 15: Linear pool KCRV relative to the reported results for Total AF, ng/g

The results are sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their standard uncertainties, *u*(*x*). The blue horizontal line denotes the candidate KCRV. The bracketing dashed lines denote the standard uncertainty of the candidate KCRV. The red data points were not included in the KCRV calculation.

DEGREES OF EQUIVALENCE (DoE)

The absolute degrees of equivalence for the participants in CCQM-K138 are estimated as the signed difference between the combined value and the KCRV: $d_i = x_i$ – KCRV. KCRV is estimated from Linear Pool Procedure of 5 participants' results. Since only 5 participants' results are entered to NICOB database to estimate KCRV and their DoE.U95 values, in order to calculate DoE.U95 values for other participants NICOB program ran a second time with all values and their values derived from this outcome. Table 16-20 below lists the numeric values of d_i, $U_{95}(d_i)$, d_i, and $U_{95}(d_i)$ for all participants in CCQM-K138 for AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs. DOE and DOE% graphs are given in figure 16-25.

Table 16: Degrees of Equivalence for AFB_1

	AFB_1, ng/g			
NMI	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
INTI	-0.90	1.16	-14.78	19.07
BAM	-0.66	1.01	-10.82	16.70
UME	-0.34	1.15	-5.68	18.98
GLHK	-0.27	1.36	-4.40	22.40
EXHM	-0.07	1.00	-1.17	16.56
NMISA	0.14	1.11	2.23	18.26
VNIIM	0.15	1.07	2.55	17.58
NIMT	0.53	1.23	8.81	20.28
KEBS	1.20	1.82	19.87	30.02

The entries in italic are results not included in the KCRV calculation

Table 17: Degrees of Equivalence for AFB_2

	AFB_2, ng/g			
NMI	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
KEBS	-0.167	0.252	-21.79	32.80
BAM	-0.107	0.162	-13.98	21.15
UME	-0.097	0.183	-12.67	23.88
INTI	-0.078	0.302	-10.12	39.37
GLHK	-0.027	0.209	-3.57	27.23
NMISA	-0.012	0.172	-1.57	22.40
NIMT	0.033	0.184	4.30	23.96
VNIIM	0.043	0.195	5.58	25.46
EXHM	0.103	0.156	13.40	20.28

The entries in italic are results not included in the KCRV calculation

Table 18: Degrees of Equivalence for AFG_1

	AFG_1, ng/g			
NMI	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
VNIIM	-0.239	0.564	-10.77	25.40
GLHK	-0.219	0.655	-9.86	29.51
BAM	-0.209	0.567	-9.42	25.54
EXHM	-0.126	0.538	-5.68	24.24
UME	-0.059	0.600	-2.65	27.02
NMISA	0.022	0.655	0.97	29.49
KEBS	0.170	0.943	7.64	42.48
INTI	0.281	0.543	12.65	24.47
NIMT	0.381	0.629	17.16	28.35

The entries in italic are results not included in the KCRV calculation

Table 19: Degrees of Equivalence for AFG_2

	AFG_2, ng/g			
NMI	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
KEBS	-0.186	0.086	-75.49	34.94
VNIIM	-0.096	0.114	-38.93	46.38
NMISA	-0.032	0.097	-12.88	39.24
GLHK	-0.026	0.114	-10.50	46.31
BAM	-0.026	0.086	-10.46	34.92
UME	-0.016	0.092	-6.36	37.56
EXHM	0.018	0.086	7.46	35.04
NIMT	0.054	0.102	22.07	41.39
INTI	0.074	0.115	30.19	46.57

The entries in italic are results not included in the KCRV calculation

Table 20: Degrees of Equivalence for Total AF

	Total AF, ng/g			
NMI	d_i	$U(d_i)$	% d_i	% $U(d_i)$
BAM	-0.992	1.494	-10.68	16.09
INTI	-0.603	1.837	-6.49	19.80
GLHK	-0.582	1.875	-6.27	20.20
UME	-0.503	1.612	-5.42	17.37
VNIIM	-0.123	1.546	-1.32	16.66
EXHM	-0.061	1.475	-0.66	15.89
NMISA	0.120	1.941	1.30	20.91
NIMT	1.018	1.689	10.97	18.20
KEBS	1.027	3.002	11.07	32.35

The entries in italic are results not included in the KCRV calculation

Figures 16-25 below graphically presents both the DOE and DOE% for AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs.

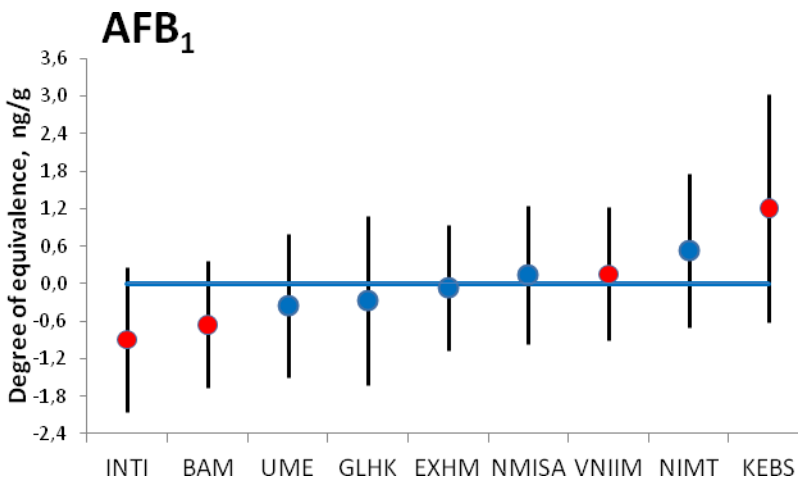

Figure 16: Absolute degrees of equivalence for AFB_1 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the absolute DoE, d, in units [ng/g]. The vertical bars correspond to ±U(di). The horizontal blue line marks the zero deviation from the KCRV.

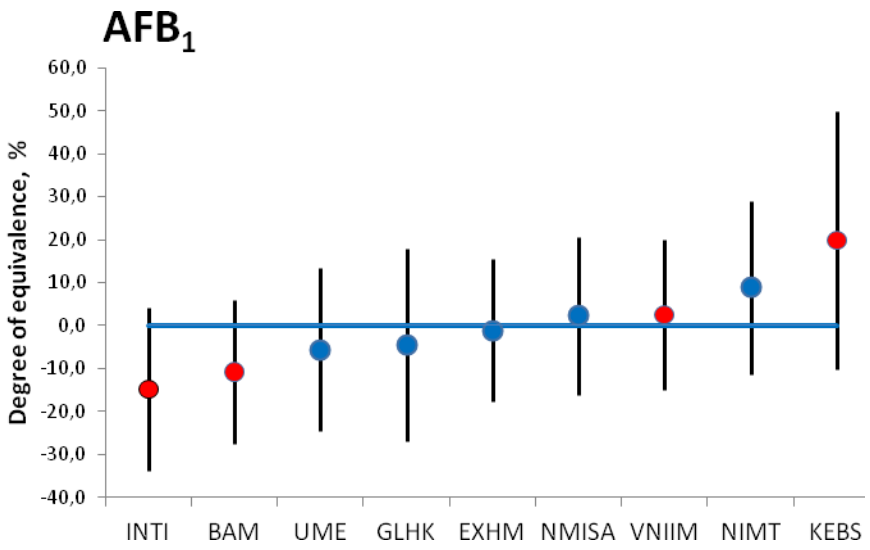

Figure 17: Relative degrees of equivalence for AFB_1 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the relative DoE, 100•d/KCRV, as percent. The vertical bars correspond to ± U(%di). The horizontal blue line marks the zero deviation from the KCRV.

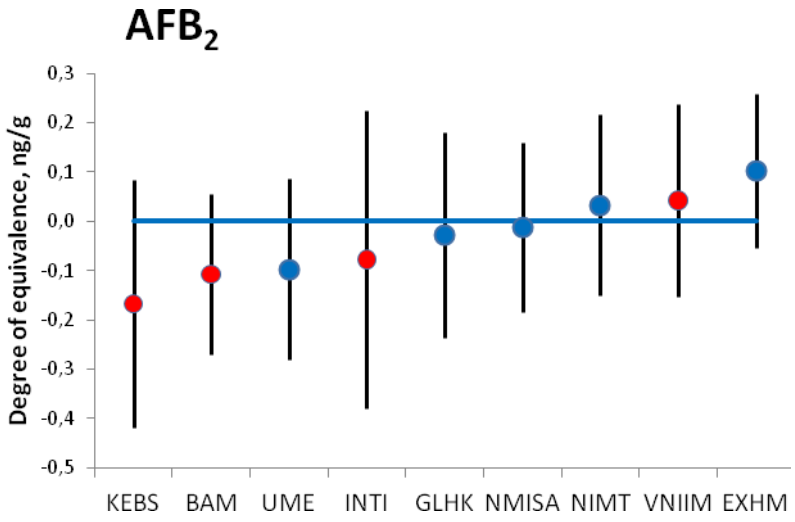

Figure 18: Absolute degrees of equivalence for AFB_2 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the absolute DoE, d, in units [ng/g]. The vertical bars correspond to ±U(di). The horizontal blue line marks the zero deviation from the KCRV.

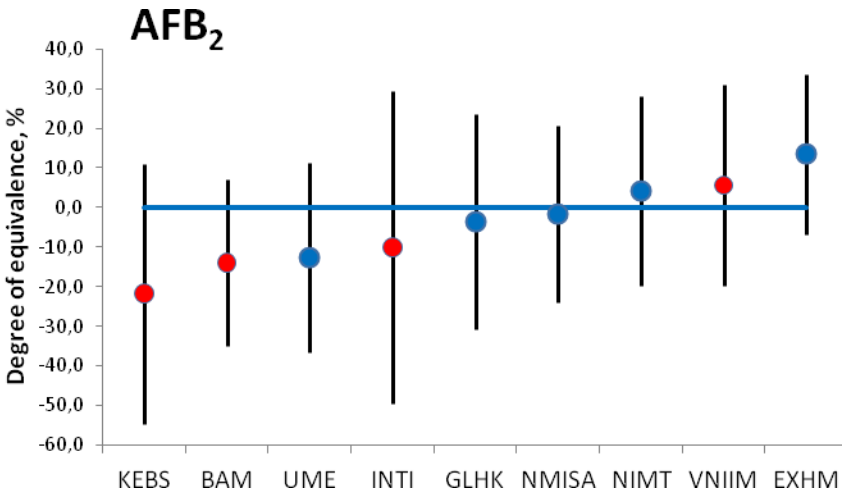

Figure 19: Relative degrees of equivalence for AFB_2 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the relative DoE, 100•d/KCRV, as percent. The vertical bars correspond to ± U(%di). The horizontal blue line marks the zero deviation from the KCRV.

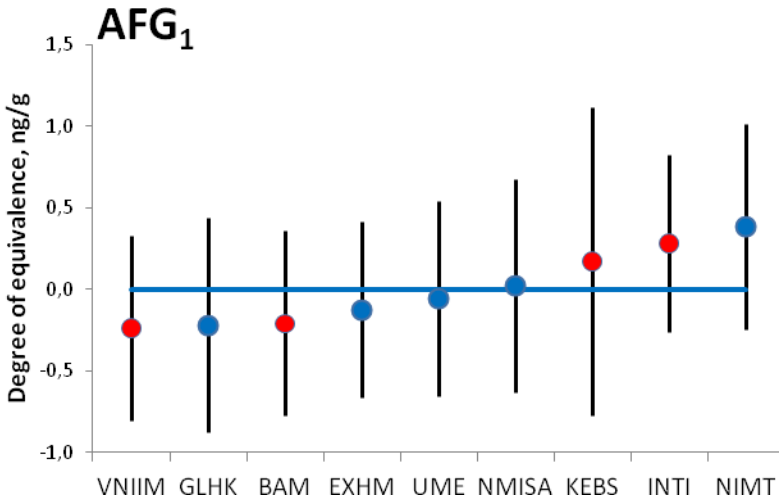

Figure 20: Absolute degrees of equivalence for AFG_1 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the absolute DoE, d, in units [ng/g]. The vertical bars correspond to ±U(di). The horizontal blue line marks the zero deviation from the KCRV.

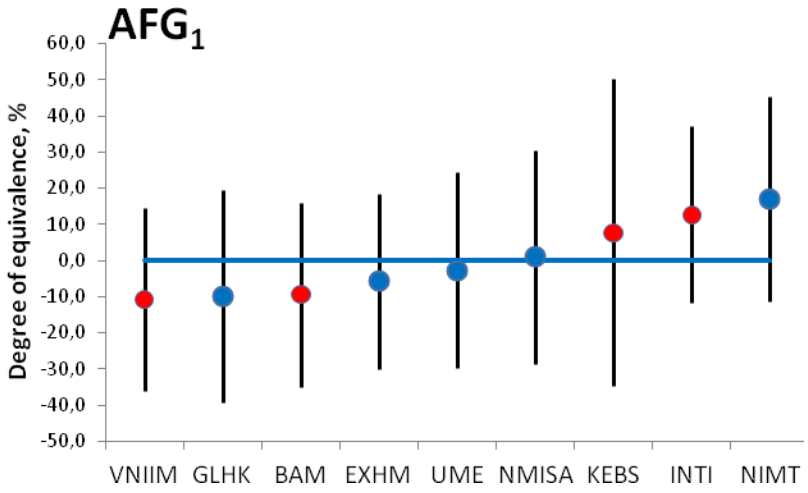

Figure 21: Relative degrees of equivalence for AFG_1 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the relative DoE, 100•d/KCRV, as percent. The vertical bars correspond to ± U(%di). The horizontal blue line marks the zero deviation from the KCRV.

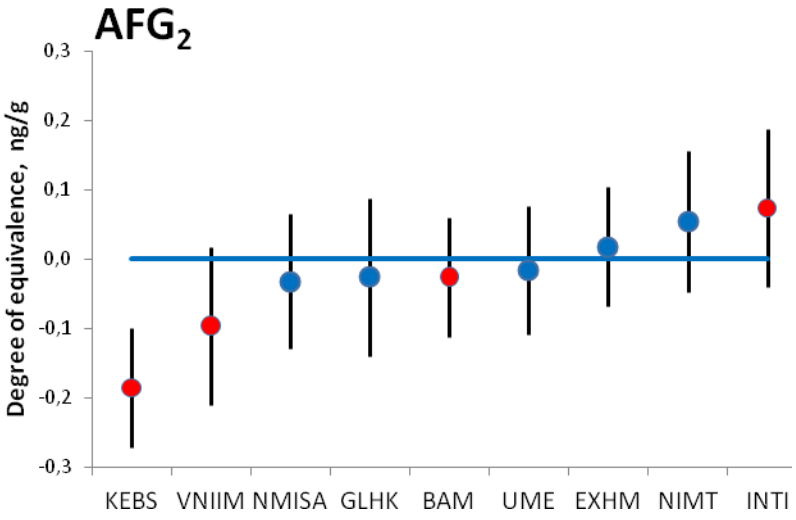

Figure 22: Absolute degrees of equivalence for AFG_2 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the absolute DoE, d, in units [ng/g]. The vertical bars correspond to ±U(di). The horizontal blue line marks the zero deviation from the KCRV.

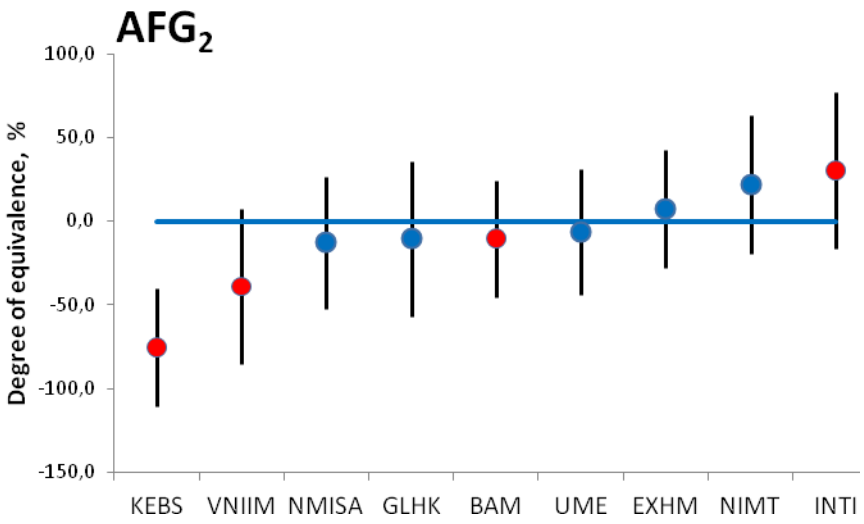

Figure 23: Relative degrees of equivalence for AFG_2 in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the relative DoE, 100•d/KCRV, as percent. The vertical bars correspond to ± U(%di). The horizontal blue line marks the zero deviation from the KCRV.

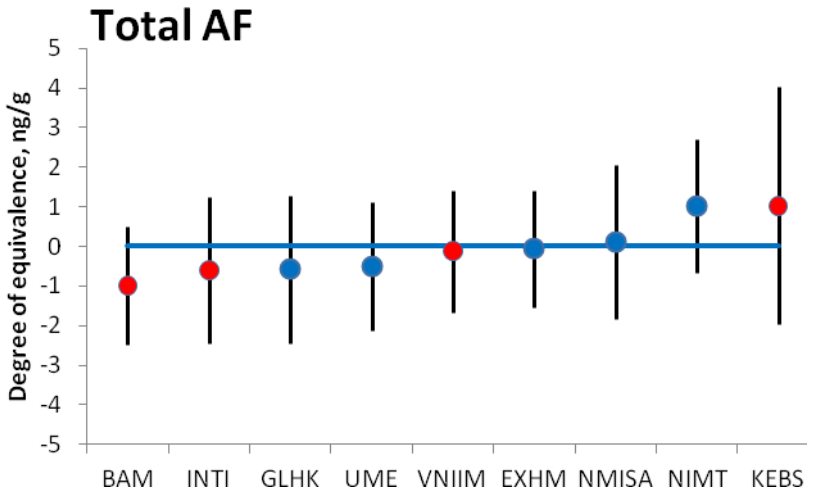

Figure 24: Absolute degrees of equivalence for Total AF in CCQM-K138.
All results are sorted by increasing value. The axis to the left edge displays the absolute DoE, d, in units [ng/g]. The vertical bars correspond to ±U(di). The horizontal blue line marks the zero deviation from the KCRV.

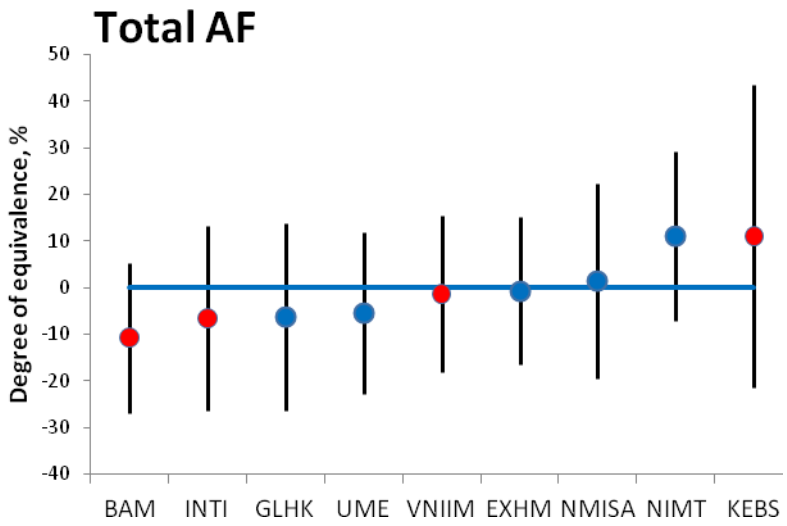

Figure 25: Relative degrees of equivalence for Total AF in CCQM-K138.

All results are sorted by increasing value. The axis to the left edge displays the relative DoE, 100•d/KCRV, as percent. The vertical bars correspond to ± U(%di). The horizontal blue line marks the zero deviation from the KCRV.

USE OF CCQM-K138 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K138 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having high polarity pKow > -2, in mass fraction range from 0.05 ng/g to 500 ng/g in dried food matrices.

It is noted that figs are a high carbohydrate form of dried foods and thus extrapolation to other types of dried food matrices should take this into account.

Core Competency Statements and CMC support

Tables E1 to E9 list the Core Competencies claimed by the participants in CCQM-K138. The information in these Tables is as provided by the participants; however, the presentation of many entries has been condensed and standardized. Details of the analytical methods used by each participant in this study are provided in Appendix F. The core competency tables are annotated to reflect the actual performance of the participants.

CONCLUSIONS

The results for CCQM-K138 represent a highly challenging set of measurands and involve very low level measurement of complex analytes in a situation where there is very limited availability of appropriate calibration materials. Participants have demonstrated capabilities to measure these analytes at levels of ranging from 5.41 ng/g to 7.27 ng/g with uncertainties ranging from 0.12 ng/g to 0.80 ng/g for AFB_1; levels from 0.60 ng/g to 0.871 ng/g with uncertainties ranging from 0.022 ng/g to 0.13 ng/g for AFB_2; levels from 1.98 ng/g to 2.6 ng/g with uncertainties ranging from 0.061 ng/g to 0.4 ng/g for AFG_1; levels from 0.06 ng/g to 0.32 ng/g with uncertainties ranging from 0.01 ng/g to 0.04 ng/g for AFG_2; levels from 8.29 ng/g to 10.31 ng/g with uncertainties ranging from 0.141 ng/g to 1.34 ng/g for Total AF.

In terms of analytical methods, most participants used immunoaffinity column cleanup and only one used SPE cleanup. All participants used liquid chromatography technique. 2 participants used florescence detector and 7 used MS detector.

Areas for improvement largely involve appropriate assessment of the traceability of the calibrants used for these measurements.

Due to the variability in results the degrees of equivalence for these analytes were reasonably large and this will need to be taken into consideration in the assessment of proposed CMCs.

ACKNOWLEDGEMENTS

The study coordinators thank all of the participating laboratories for providing the requested information during the course of these studies. We would like to thank to Lindsey Mackay, Michael Nelson and Katrice Lippa for their invaluable contributions to the report.

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APPENDIX A: Call for Participation

From: Lindsey.Mackay@measurement.gov.au
Date: 25.11.2015 21:50

Dear OAWG colleagues

Attached please find all of the documentation for our next Track C key comparison for aflatoxins in fig. Please return registration forms to UME by 4 December and contact me if you have any questions about the comparison.

Many thanks

Lindsey

Attachments: CCQM K138/P174_ Registration form.docx
CCQM K138/P174 Technical protocol.docx
CCQM K138 Core Competency Table .doc
CCQM K138/P174 Report Form.xlsx
CCQM K138/P174 Sample Receipt Confirmation form

APPENDIX B: Protocol

TÜBİTAK UME	**CCQM-K138 and P174** **Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig** **TECHNICAL PROTOCOL**	TÜBİTAK UME

CCQM-K138 and P174

Key and Pilot Comparisons on

"Determination of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs) in Dried Fig"

Call for Participants and Technical Protocol

(February 24, 2016)

1. Introduction

Dried fig can be consumed directly or as fig paste/slurry in desserts and candies [1]. It is considered a healthy food as its nutritional value is high. It has highly alkaline property, which makes it useful in balancing the pH of fibre. It is a rich source of potassium and calcium, which is important in helping to regulate blood pressure and as an alternative to dairy products for the people who have allergies. Calcium and potassium are also important in preventing osteoclasis. Dried fig contains good level of magnesium, iron, copper and manganese. Tryptophan in fig induces good sleep and helps in preventing sleeping disorders like insomnia. It helps to reduce the risk of breast cancer and blood cholesterol level [2].

The production of dried fig involves some unique agricultural practices such as ripening, harvesting and sun-drying. These practices present significant risk of fungal infection and subsequent mycotoxin contamination. National and international institutions and organization such as the European Commission (EC), the US Food and Drug Administration (FDA), the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) have recognized that mycotoxins have potential risk to human and animal health. Regulations have been established in many countries to protect consumers from their harmful effects. The European Union (EU) has introduced severe limits in many products for major mycotoxin classes as high risk of contamination (Commission Regulation No.1881/2006). The European legislation has set maximum limits for various mycotoxins in food and feed, including Aflatoxins (B_1, B_2, G_1 and G_2), which are extremely toxic, carcinogenic, tetratogenic and hepatotoxic.

Exported products to the EU are sometimes rejected and withdrawn because of high levels of aflatoxins. Alert notifications are published weekly on the internet to inform the member states by a Rapid Alert System as the monitoring is very important for consumer protection and producers of raw products prior to transport or processing [3-8].

Turkey, USA, Iran and Mediterranean countries are the major producers of dried fig. Half of the international trade in dried figs is conducted by Turkey, which produces 60 % of the total worldwide supply. Therefore, the sustainable export of dried figs has great significance for the Turkish agricultural economy. To ensure its sustainability, it is necessary to satisfy internationally accepted sanitation and hygiene standards during production, storage and delivery to consumers. To this end. aflatoxin contamination in exported figs should be monitored through reliable and traceable measurement methods. The traceability of aflatoxin measurement results can be achieved through the use of pure and matrix certified reference material. However, for the determination of aflatoxins in dried fig, such certified reference materials are not yet available. There is a lack of certified reference materials (CRMs) for use by routine testing laboratories in method validation and as quality controls. In addition, commercial proficiency testing (PT) programmes, commonly participated in by routine testing laboratories, make use of consensus results instead of metrologically traceable assigned values to evaluate the performance of the participating laboratories. The proposed study material is a candidate certified reference material for the determination of aflatoxin (B_1, B_2, G_1, G_2 and total) levels in dried fig [9-12].

The study was first proposed as a key comparison and presented at the EURAMET TC-MC SCOA meeting in Malta in 2015. During the meeting, three NMIs / DIs expressed interest to participate in the study. Hence, the meeting recommended that the study should proceed and be presented during the CCQM OAWG meeting by EURAMET. The study was subsequently presented at the CCQM OAWG meeting in April 2015. CCQM OAWG members from other RMOs would also be invited to participate in the study. During the meeting, five NMIs/DIs expressed interest to participate in the study. An approval was subsequently obtained from the CCQM OAWG Chair to organize this study as a Track C key comparison and as a pilot study.

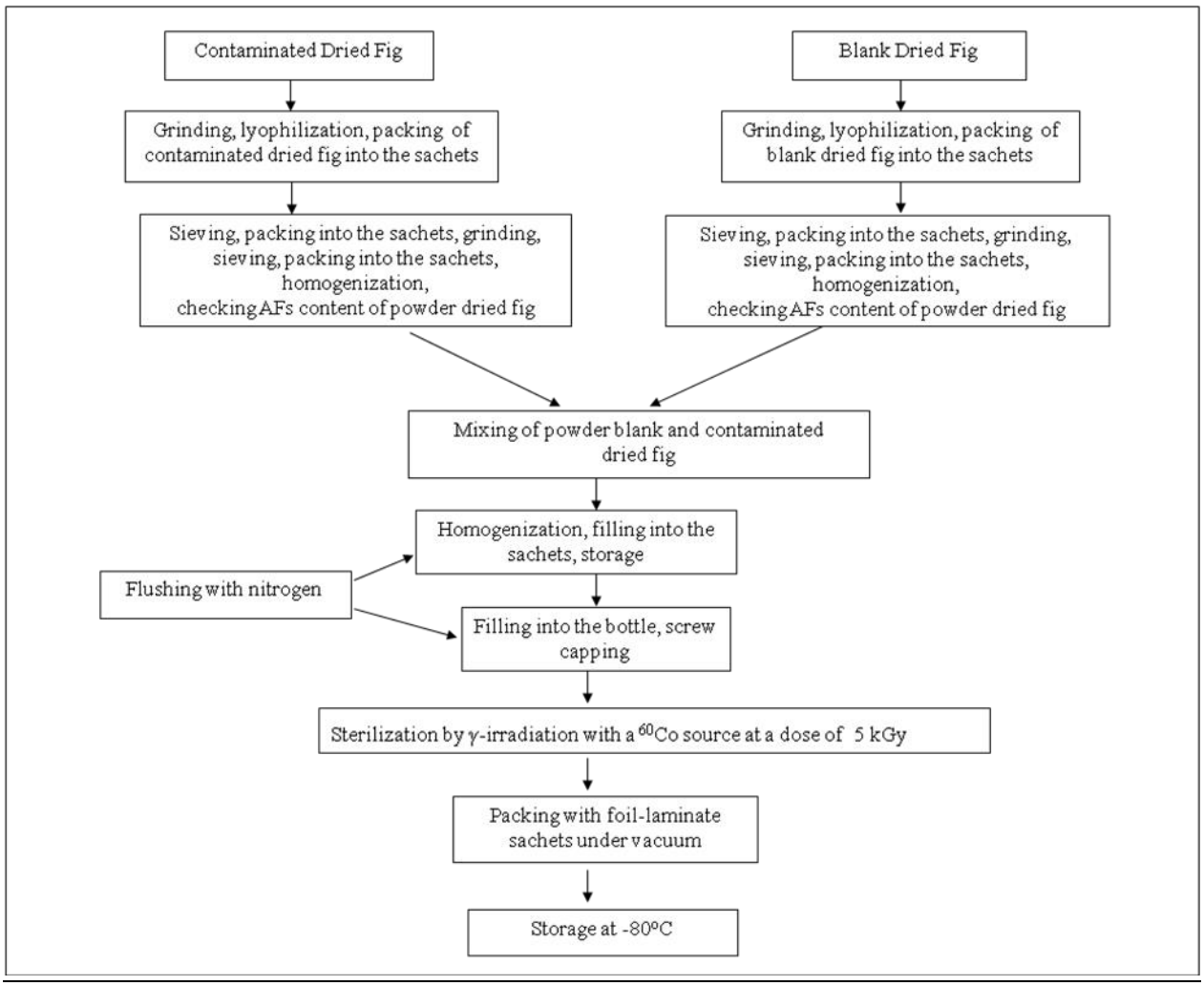
2. Test material

The test material is a candidate material for a dried fig certified reference material (CRM). The mass fraction of aflatoxin (B_1, B_2, G_1, G_2 and total) in dried fig will be certified in the near future. The results of this comparison will be mentioned in the certification document.

2.1. Preparation of Study Samples by TUBITAK UME

The raw material to be used in this study was obtained from the province of Aydın which supplies 70-75 % of all dried figs in Turkey. Dried fig material was stored at -18 ºC until processing. Raw material was blended by a blade mixer, dehydrated using a freeze dryer, grounded, sieved and packed as 165 g in bottles. The bottles were packed with foil-laminate

sachets under vacuum. All the sample bottles were stored at room temperature (-80 ± 3) °C inside prior to distribution or use. Steps in the preparation of study samples are given in Scheme 1 below.



Scheme 1. Preparation steps of study samples

2.2. Homogeneity and Stability Testing of samples

The homogeneity of the material was investigated by analyzing 12 bottles selected from 500 bottles. The bottles were randomly and stratified selected. Three subsamples (6 g) were taken from each bottle for homogeneity. The data were treated with ANOVA. The samples were measured in a random order under repeatability conditions. The data were technically scrutinised and statistically evaluated according to ISO Guide 30 to 35. The preservatives were found to be sufficiently homogeneous in the study material. The relative standard uncertainties due to between-bottle inhomogeneity for AFB_1, AFB_2, AFG_1, AFG_2 and total AFs were found to be

2.28 %, 4.61 %, 4.31 %, 4.68 % and 2.05 %, respectively. The results of the homogeneity study are summarized in Table 1.

Table 1. Results of the homogeneity assessment for target aflatoxin (B_1, B_2, G_1, G_2 and total) in dried fig

	AFB_1	**AFB_2**		**AFG_1**	**AFG_2**	**Total AFs**
$S_{between}$ (%)	2.27	$MS_{between} < MS_{within}$		7.46	4.00	1.52
u^*_{bb} (%)	2.28	4.07		4.31	4.68	2.05
u_{bb} (%)	2.28	4.07		7.46	4.68	2.05
RSD	7.67	8.40		15.67	15.57	6.76
F	1.29	0.63		1.86	1.21	1.16
F-critic	2.22					
P-value	0.29	0.79	0.10		0.33	0.36

When $MS_{between}$ is smaller than MS_{within}, $S_{between}$ cannot be calculated. This does not prove that the material is perfectly homogeneous, but only indicates that study set-up was not good enough to quantify heterogeneity. Instead of S_{bb}, u^*_{bb}, the heterogeneity that can be hidden by the method repeatability, is calculated.

A four week isochronous study was performed to evaluate stability of candidate reference material during transport. The bottles were selected using a random stratified sample picking computer programme. Two subsamples (50 g) were taken from each bottle for stability tests. For a short-term stability study, -20 ºC and 4 ºC were selected as test temperatures. The selected test periods were 0, 1, 2, 3 and 4 weeks. After the indicated storage periods. the samples were stored at -80 °C until analysis. For each test temperature and test periods, 2 bottles were analyzed. Each sample bottle was analyzed in duplicate. Two replicates of each bottle were analyzed randomly under repeatability conditions. All data were evaluated for short-term stability test according to ISO Guides 30 to 35. Regression lines were calculated to detect possible degradation. Although the slope was found to be indistinguishable from zero for storage temperatures of -20 ºC and 4 ºC, a significant slope was found when the samples were stored at -80 ºC. The uncertainty of the short-term stability (u_{sts}) can be assumed to be negligible if the sample shipment is carried out with cooling elements or on dry ice. Results of the short-term stability study are summarized in Table 2.

Table 2. Results of the short-term stability assessment for target Aflatoxin (B_1, B_2, G_1, G_2 and total) in dried fig

	u_{sts} (%) (-20 °C)	u_{sts} (%) (+4 °C)
AFB_1	1.20	1.20
AFB_2	1.22	1.36
AFG_1	3.23	2.46
AFG_2	2.88	2.31
Total AFs	1.38	1.22

The same method (HPLC-FLD) was used for the homogeneity and short-term stability measurements.

For the long-term stability study, -20 ºC and 4 ºC remained as the selected test temperatures, while the test periods of 2, 4, 6 and 9 months were used. All data will be evaluated for long-term stability test according to ISO Guides 30 to 35 until the deadline for submission of results.

Different amount of subsamples was used for the minimum sample intake study.

Table 3. Results of the minimum sample intake study for target Aflatoxin (B_1, B_2, G_1, G_2 and total) in dried fig

Analyte	**RSD**			
	2 g	**4 g**	**6 g**	**50 g**
AFB_1	20	19	9	9
AFB_2	13	13	7	4
AFG_1	16	21	15	15
AFG_2	22	22	18	14

Results of the minimum sample intake study are summarized in Table 3. According to results, minimum sample intake is recommended as at least 6 g.

In the same day (within day repeatability), the relative standard deviation of measurement results of AFB_1, AFB_2, AFG_1, AFG_2 and total AFs were found 7.75 %, 8.74 %, 16.2 %, 16.7 % and 7.09 %, respectively for 6 g sample intake.

3. Measurands

The measurands to be determined are the mass fractions of aflatoxin (B_1, B_2, G_1, G_2 and total) in dried fig. The structures of Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) are given in Figure 1.

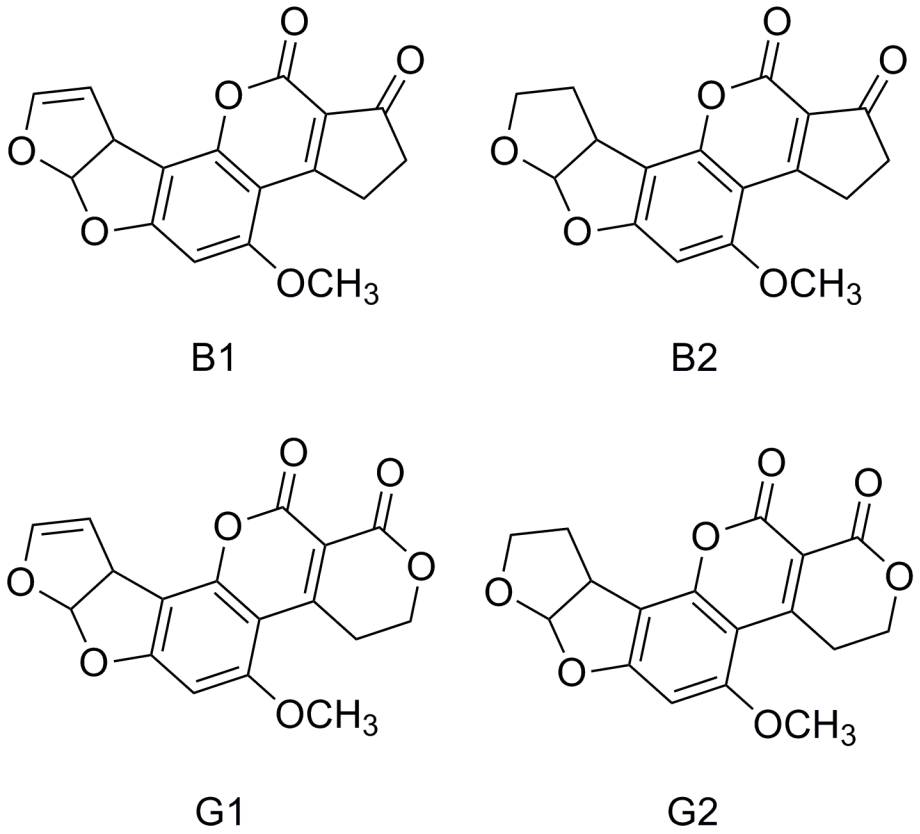


Figure 1. Structures of Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2)

The nominal values of Aflatoxin B_1 are between mass fractions of 3 ng/g to 7 ng/g. Aflatoxin B_2 between mass fractions of 0.3 ng/g to 1 ng/g, Aflatoxin G_1 between mass fractions of 1 ng/g to 3 ng/g, Aflatoxin G_2 between mass fractions of 0.08 ng/g to 0.3 ng/g, total Aflatoxin between mass fractions of 6 ng/g to 9.5 ng/g.

Analytes and those nominal values in the candidate reference material are also given in Table 3.

Table 3. AFB_1, AFB_2, AFG_1, AFG_2 and Total AFs Expected Mass Fractions

Analytes	Mass Fraction (ng/g)
AFB_1	3-7
AFB_2	0.3-1
AFG_1	1-3

AFG_2	0.08-0.3
Total AFs	6-9.5

4. Handling and storage

To avoid any decomposition, the samples should be kept sealed until they are used. They should be stored at the temperature from -20 ^{o}C to +4 ^{o}C in its original bottle, tightly capped and not exposed to intense direct light and ultraviolet radiation. The samples should be opened carefully and the measurement should be carried out immediately after the samples are opened.

5. Distribution

The participants will be informed of the date of dispatching of samples. Each participant will receive 2 units candidate reference material (HDPE bottles into aluminium sachet containing about 165 g of powder dried fig).

Participants are required to acknowledge the receipt of the sample. and return the receipt to TUBITAK UME by e-mail. If there is any damage on the sample, TUBITAK UME will send a substitute sample on request. A Sample Receipt Confirmation Form as a receipt form will be distributed to the participants. After receiving the sample, it should be kept at a temperature between -20 and +4 °C.

6. Methods/procedures

Each participant is encouraged to use their typical analytical method. Please include a full description of your method of analysis when reporting the results. For this purpose, a "Report Form" will be sent to the participants. NMIs or officially designated institutes are welcome to participate in this comparison. If ID-MS methods are used, the source of isotopically labeled spike material used should be reported.

7. Analysis and Uncertainty Evaluation

The units should be stored between -20 to +4 °C and should be equilibrated to room temperature before analysis for 2 hours.

Before opening the sample, the material must be homogenised by shaking the container for 2 min to prevent possible clumping. The analysis should be conducted with a recommended sample size of at least 6 g.

The report should comprise a brief description of the measurement method (including sample preparation) as well as a brief description of quality assurance measures. The calibration solutions and the individual results (for each parameter analyzed) should be reported in ng/g. All

results must be linked to the TUBITAK UME sample identification number (unit number) and to the date of the analyses.

Each participant laboratory should use an appropriate approach following the ISO/GUM and the approach used to derive the uncertainty budget must be briefly described in the report. Each variable contributing to the uncertainty of the result should be identified and quantified in order to be included in the combined standard uncertainty of the result. A full uncertainty budget must be included in the report.

Every participant laboratory should use its usual aflatoxin calibrants and establish their traceability.

8. Reporting and submission of results and core capability assessment

The result should be reported as the mass fraction of each measurand, mean of from two sample, to TUBITAK UME, accompanied by a full uncertainty budget. The result should be submitted using the attached Report Form.

Furthermore, all participants in this comparison are required to complete a Core Capability Table for the measurement technique they used. Templates for the appropriate techniques will be sent to the registered participants when the sample is distributed. The filled-out table should be submitted together with the measurement result.

Please complete and submit the attached Report Form and the Core Capability Table to TUBITAK UME (E-mail: ahmetceyhan.goren@tubitak.gov.tr) by e-mail before the scheduled deadline.

The report must include:

- ✓ Result should be reported as a value of independent measurements of two bottles of comparison sample with corresponding standard and expanded uncertainty.
- ✓ The value of the results and their associated standard uncertainties must be expressed in ng/g.
- ✓ If the final result has been calculated from more than one method, the individual results from the contributing methods must also be reported.
- ✓ A detailed description of the applied analytical procedure including the sample preparation and calibration methods.
- ✓ Participants are asked to provide information about their metrological traceability.
- ✓ Each participant should make an assessment of the measurement uncertainty. Each variable contributing to the uncertainty of the result should be identified and quantified in order to be included in the combined standard uncertainty of the results. A full uncertainty budget must be reported, as part of the results.
- ✓ All cells in all sheets (Result Reporting Form and Method Information) in Annex 2 “Report Form” should be filled out in the excel file that will be provided in electronic form by TUBITAK UME.

9. KCRV

Each laboratory should use a properly validated method, calibration standards with a metrologically traceable assigned purity value (CRM or material where its purity has been suitably assessed by the participant) according to criteria established by the CCQM OAWG for the inclusion of results in the calculation of the KCRV. Exclusion of data points in the KCRV calculation will be using a sound of metrological basis. On the basis of this information, appropriate estimators and uncertainty evaluation for the KCRV will be proposed (see for reference the "OAWG Practices and Guidelines" document). It is expected that it is most likely that each reference value will be the median of the submitted data from NMIs and officially designated institutes, though it will be decided after discussion in CCQM OAWG meeting. If any participant submitted individual results by multiple methods, their best result (*i.e.*, with the smallest uncertainty) will be selected to calculate the reference value. The final decision regarding the assignment of a KCRV and its uncertainty for CCQM-K138 and P174, will be taken after discussion in the November 2016 CCQM OAWG meeting.

10. How Far Does the Light Shine?

This Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity ($pK_{ow} > -2$) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.

11. Program schedule

- ❑ Draft protocol and conformation: October 2015
- ❑ Call for participation: November 2015
- ❑ Deadline for registration: December 2015
- ❑ Distribution of study sample: February 2016
- ❑ Deadline for submission of results: 30th September 2016
- ❑ Presentation/initial discussion of results: November 2016 CCQM OAWG
- ❑ Draft A report: December 2016

12. Participants

Participation is open to all interested NMIs or officially designated institutes that can perform the determination.

13. Coordinating laboratory

The CCQM-K138 and P174 are coordinated by TUBITAK UME. TUBITAK UME takes all responsibilities for the development and operation of the key comparison, including preparation and distribution of samples, initial data analysis and evaluation of results to facilitate OAWG discussions, draft reports, and communications with participants.

14. Registration

Please complete and return the attached registration forms to TUBITAK UME (E-mail: ahmetceyhan.goren@tubitak.gov.tr) for the participation. Successful registration will be notified by e-mail. Please register no later than **04 December 2015**.

15. Confidentiality

The participating laboratories will receive the reports giving all results for assessment/comments. The participating laboratories will be identified in the reports. The key comparison is conducted in the belief that participants will perform the analysis and report results with scientific rigor. Collusion between participants or falsification of results is clearly against the spirit of this study. Once approved by the OAWG, this report will be available on the open access section of the BIPM website. Participants may not publish any such data until the key comparison report has been published on the KCDB.

16. Contact

For any enquiries, participants may wish to contact the persons from coordinating laboratory are as follows:

TUBITAK Ulusal Metroloji Enstitusu (UME)

Dr. Ahmet Ceyhan GOREN

E-mail: ahmetceyhan.goren@tubitak.gov.tr

Phone: 00 90 262 679 50 00 (6102)

Fax: 00 90 262 679 50 01

17. References

[1] Bircan. C.. Barringer. S.A.. Ulken. U.. Pehlivan. R.. 2008. ”Aflatoxin levels in dried figs. nuts and paprika powder for export from Turkey”. International Journal of Food Science and Technology. 43. 1492-1498.
[2] http://www.figyork.com/production.html.
[3] htts://ec.europa.eu/food/food/rapidalert/report2007-en.pdf.
[4] http://ec.europa.eu/food/food/rapidalert/docs/report2009_en.pdf.
[5] http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2010_en.pdf.
[6] http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2011_en.pdf.
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APPENDIX C: Registration Form

	CCQM-K138 and P174 **Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**	
	REGISTRATION FORM	

Please complete the following:

Name of Institute : _____

Acronym of Institute (if available) : _____

Name of Laboratory/Department _____

Name of Contact Person : _____

Designation : _____

E-mail Address : _____

Telephone Number : _____

Fax Number : _____

Postal Address : _____

Postal Code : _____

Country : _____

Date : _____

Please tick the appropriate boxes.

1) **We would like to register for the following measurements;**

Analytes				
Aflatoxin B_1 (AFB_1)	**Aflatoxin B_2 (AFB_2)**	**Aflatoxin G_1 (AFG_1)**	**Aflatoxin G_2 (AFG_2)**	**Total Aflatoxin (Total AFs)**
K138 ☐ P174 ☐	K138 ☐ P174 ☐	K138 ☐ P174 ☐	K138 ☐ P174 ☐	K138 ☐ P174 ☐

2) **Do you require a special custom permit for the samples to be sent to your laboratory?**

☐ Yes ☐ No (If yes, please give the details in a separate paper.)

Please note that any import taxes or charges, imposed on the material during transportation, shall be met by the participating laboratory.

Kindly complete and return this form by e-mail or fax no later than 04 December 2015 to:

Dr. Nilgun TOKMAN
TUBITAK UME
Gebze Yerleskesi P.K. 54 41470
Gebze-Kocaeli/Turkey
E-mail: nilgun.tokman@tubitak.gov.tr
Phone: 00 90 262 679 50 00 (6203)
Fax: 00 90 262 679 50 01

Dr. Ahmet Ceyhan GOREN
TUBITAK UME
Gebze Yerleşkesi P.K. 54 41470 Gebze-Kocaeli/Turkey
E-mail: ahmetceyhan.goren@tubitak.gov.tr
Phone: 00 90 262 679 50 00 (6201)
Fax: 00 90 262 679 50

If you do not receive an acknowledgement for your registration from us within 4 working days, please send us an email.

APPENDIX D: Reporting Form

The original form was distributed as an Excel workbook. The following are pictures of the relevant portions of the workbook's two worksheets.

"Result Reporting Form" worksheet

ANNEX-2

UME

Mass Fractions of Aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in Dried Fig

CCQM-K138 and P174

RESULT REPORTING FORM

Please use this excel sheet for reporting. Please don't use different form.
Report should be send to ahmetceyhan.goren@tubitak.gov.tr electronically until 30th September 2016.
Please write all requested information in appropriate section completely.
Additional information can be given in remarks section or a separate sheet if necessary. .

Participant Information:

Name of Institute:	
Postal Address:	
Name of Contact Person:	
Name of Analyst(s):	
Telephone / Fax::	
E-mail Address:	
Date of Reporting:	..../..../2016
CCQM-K138	Yes ☐ No ☐
CCQM-P174	Yes ☐ No ☐

Results:

"Result Reporting Form" worksheet (continued)

Analytes	Sample Name/ Unit No	Mass Fraction (ng/g)	Mean Value (ng/g)	Combined Standard Uncertainty* (ng/g)	Coverage Factor* (k)	Expanded Uncertainty (ng/g)
Aflatoxin B_1						
Aflatoxin B_2						
Aflatoxin G_1						
Aflatoxin G_2						
Total Aflatoxin						
Remarks						

Measurement Equation and Uncertainty Budget

Please give the measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

Please describe individual uncertainty contributions and estimation of uncertainties for each factor. Please give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Additional information, observations or comments

"Method Information" Worksheet

Method Information

Name of Institute:	
Postal Address:	
Name of Contact Person:	
Name of ame of Analyst(s):	
Telepfone / Fax:	

Sample Preparation

Used method:	
Amount of sample:	
Extraction solvent:	
Purification solvent:	
Volume of extraction solvent:	
Sample preparation procedure (Please explain briefly):	
Remarks:	

Calibration

	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2
Name of calibrants or reference materials:				
Batch number of calibrants or reference materials:				
Number of calibrants:				
Traceability				
Calibration type				
Concentration of stock solution (as a mass fraction)				
Concentration of calibrants (as a mass fraction)				
LOD/LOQ (as a mass fraction)				
Information of quality control sample				
Remarks				

"Method Information" Worksheet (continued)

Method and Instrument Information

Measurement technique	
Measurement method	
Instrument parameters	
Analysis conditions (such as mobile phase, detector condition, column properties, column temperature, column pressure, analysis time, flow rate, injection volume)	
Others:	

*NA : It can be used for not applicable area.

Remarks: Please write your comment regarding issue such interferences, accuracy, small changes during the measurement.

Date:

APPENDIX E: Core Competency Tables

CCQM OAWG: Competency Template for Analyte(s) in Matrix

Instructions:

- In the middle column place a tick, cross or say the entry is not applicable for each of the competencies listed (the first row does not require a response)
- Fill in the right hand column with the information requested in blue in each row
- Enter the details of the calibrant in the top row, then for materials which would not meet the CIPM traceability requirements the three rows with a # require entries.
-

Table E.1. Core Competencies Demonstrated in CCQM-K138 by BAM

CCQM-K138	***BAM***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick. cross. or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		calibration solutions (Biopure. RomerLabs): B1: 16192B, B2: L15483A., G1: L15331C, G2: L15391A, 13C17-Afla-Mix: I15383M
Identity verification of analyte(s) in calibration material.#	✓	Mass spectrometric investigations (MRM. fragmentation pattern)
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	Commercial certified standard solutions used, not suited for e.g. qNMR (purity assessment).
For calibrants which are a calibration solution: Value-assignment method(s).#	✓	Certified standard solutions used. Purities of calibration standards were independently confirmed by LC-MS measurements (scan mode; ESI+/-). The specified aflatoxin contents of the used certified standard solutions were cross checked by certified standards of different lot numbers (same provider) and certified standard solutions of a second provider.
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, internal standard, mass spec ion ratios (quantifier/qualifier)
Extraction of analyte(s) of interest from matrix	✓	shaking extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	IAC (Aflastar™)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	-
Analytical system	✓	HPLC-MS/MS

Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS; six-point calibration; linear regression
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	-
Other	N/A	-

Table E.2. Core Competencies Demonstrated in CCQM-K138 by EXHM

CCQM-K138	***EXHM***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		ERM AC 057, 058, 059, 060 solutions in-house Aflatoxin solutions
Identity verification of analyte(s) in calibration material.#	✓	LC-MS/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	mass balance (LC-UV,KF titration, ICPMS) qNMR
For calibrants which are a calibration solution: Value-assignment method(s).#	✓	IRMM CRMs: used certified values checked versus qNMR analysis of in house pure materials: UV-Vis (according to EN 14123)
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	✓	Liquid/liquid, ASE
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	immunoaffinity column
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	--
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS, exact matching matrix matched, single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	--
Other	N/A	--

Table E.3. Core Competencies Demonstrated in CCQM-K138 by GLHK

CCQM-K138	***GLHK***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration Solutions Used: Aflatoxin B1 : IRMM ERM – AC057 Aflatoxin B2 : IRMM ERM – AC058 Aflatoxin G1 : IRMM ERM – AC059 Aflatoxin G2 : IRMM ERM – AC060
Identity verification of analyte(s) in calibration material.#	N/A	--
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	--
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	--
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and ion ratio of mass spectrometric analysis
Extraction of analyte(s) of interest from matrix	✓	Liquid/solid extraction by high speed homogenizer – 2 times extraction by water and followed by 3 times extraction by 80% methanol
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Cleanup - by immunoaffinity column
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	--
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	Quantification mode used - Isotope Dilution Mass Spectrometry Calibration mode used – Standard addition
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	--
Other	N/A	--

Table E.4. Core Competencies Demonstrated in CCQM-K138 by INTI

CCQM-K138	***INTI***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Pure material. Fluka AG. Aflatoxins B1, B2, G1 and G2 from Aspergillus Flavus. Aflatoxin B1 Cat. Code: 5032 Batch 2216541280. Aflatoxin B2 Cat. Code: 5033 Batch 202621578. Aflatoxin G1 Cat. Code:5035 Batch 219939181. Aflatoxin G2 Cat. Code: 5036 Batch 219940181.
Identity verification of analyte(s) in calibration material.#	✓	Spectrophotometric method (AOAC 971.22).
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	One solution of each aflatoxin was prepared to obtain 4 stock solutions of 8-10 ug/ml in acetonitrile. These solutions were verified using an Spectrophotometric method (AOAC 971.22). After the measurement of the stock solution at 350nm. it was adjusted the purity of each calibration solution. The assignment of purity was determinated following the next equation: $\%\ \text{purity} = \frac{cc_{\text{standard stock}} \times 10\text{ml} \times 5000\text{ul} \times 100}{50\ \text{ul} \times 1000\ \text{ug/mg} \times 10\ \text{mg}}$
For calibrants which are a calibration solution: Value-assignment method(s).#	**N/A**	Indicate how you established analyte mass fraction in calibration solution
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time with external standard
Extraction of analyte(s) of interest from matrix	✓	The analyte is extracted using solvent extraction (MeOH+H20) (8+2 v/v)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Cleanup with immunoaffinity column. Chromatographic Separation with LC.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Post-column derivatization involving bromination. (Kobra Cell)
Analytical system	✓	LC-FD (Liquid Chromatography with fluorescence detector.
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) external standard b) 5 points calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	We do not use any verification. Verification is not necessary due specificity of cleanup separation used in the method.
Other	**N/A**	Indicate any other competencies demonstrated.

Table E.5. Core Competencies Demonstrated in CCQM-K138 by KEBS

CCQM-K138	***KEBS***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration solution used AFB1 Source FERMENTEK Lot# AF017 AFB2 Source TRILOGY ANALYTICAL LABORATORY Lot# 141104-070 AFG1 Source TRILOGY ANALYTICAL LABORATORY Lot# 150305-070 AFG2 Source TRILOGY ANALYTICAL LABORATORY Lot# 150309-070
Identity verification of analyte(s) in calibration material.#	√	UV/VIS with Acetonitrile as solvent
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	--
For calibrants which are a calibration solution: Value-assignment method(s).#	√	UV/VIS with Acetonitrile as solvent and application of Beer's Law
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	Retention time
Extraction of analyte(s) of interest from matrix	√	Extraction by using a shaker and 80% Methanol
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	Immunoaffinity columns used
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	√	Electro-chemical derivertization
Analytical system	√	HPLC with FL detection
Calibration approach for value-assignment of analyte(s) in matrix	√	a)External standard b) 5- point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	--
Other	N/A	--
NOTE: KEBS results for AFG2 was not consistent with the KCRV and had a DoE that did not cross zero. The specific reason for this deviation was not identified although KEBs did not use appropriate traceable calibrants for all of the analytes.		

Table E.6. Core Competencies Demonstrated in CCQM-K138 by NIMT

CCQM-K138	***NIMT***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		ERM-C057, ERM-C058, ERM-C059, ERM-C060
Identity verification of analyte(s) in calibration material.#	N/A	--
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	--
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	Gravimetric
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The analytes in the samples were identified against ERM-CO57, ERM-CO58, ERM-CO59 and ERM-CO60 standards by comparing their retention times and m/z of LC-MS/MS.
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction using 70:30 MeCN: water with 20 mL of extraction solvent: 10 grams sample
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Immunoaffinity column (IAC)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	Indicate chemical transformation method(s), if any, (i.e., hydrolysis, derivatization. other)
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS. b) 6-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	--
Other	N/A	--

Table E.7. Core Competencies Demonstrated in CCQM-K138 by NMISA

CCQM-K138	***NMISA***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		IRMM ERM individual aflatoxin B1, B2, G1 and G2 ERM solutions respectively: ERM_AC057AFB1 ILM010 Lot I15231A ERM_AC058AFB2 ILM011 Lot I15345B ERM_AC059AFG1 ILM012 Lot I15345A ERM_AC060 AFG2 ILM013 Lot I15232G
Identity verification of analyte(s) in calibration material.#	✓	Verification by comparison of HPLC-FLD. UPLC-MS/MS Retention time, FLD excitation-emission wavelength, multi-reaction monitoring (MRM) ion ratio transitions unique to the toxins using IRMM ERMs and other commercial standards of the mycotoxins (Biopure™ and Trilogy™)
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	-
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	-
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Identification by comparison of HPLC-FLD, UPLC-MS/MS Retention time. FLD excitation-emission wavelength., multi-reaction monitoring (MRM) ion ratio transitions unique to the toxins using IRMM ERMs and other commercial standards of the mycotoxins (Biopure™ and Trilogy™)
Extraction of analyte(s) of interest from matrix	✓	Methanol: Water (80:20)saline solid-liquid extraction of the dried fig powder with shaking 60 min.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Immunoaffinity clean-up (VICAM Aflatest)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	-
Analytical system	✓	UPLC-ESI-MS/MS, HPLC-FLD independent check.
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) double IDMS, standard addition, external standard b) 9-point std addition; 6-point external calibration; 3 brackets dIDMS.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	-
Other	N/A	-

Table E.8. Core Competencies Demonstrated in CCQM-K138 by VNIIM

CCQM-K138	***VNIIM***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration solution. RM from Biopure
Identity verification of analyte(s) in calibration material.#	✓	LCMS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	-
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	From certificate of analysis
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	✓	Sonication
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	-
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS, single point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	-
Other	N/A	-

Table E.9. Core Competencies Demonstrated in CCQM-K138 by TUBITAK UME

CCQM-K138	***UME***	**Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig**
Scope of Measurement: This Track C Key Comparison will demonstrate capabilities for low molecular mass (100 g/mol to 500 g/mol) analytes of high polarity (pK_{ow} > -2) at the 0.05 ng/g to 500 ng/g mass fraction range in dried food matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance. commercially available from SIGMA: Aflatoxin B1 from aspergillus flavus A6636; Aflatoxin B2 A9887; Aflatoxin G1 A0138; Aflatoxin G2 A0263
Identity verification of analyte(s) in calibration material.#	✓	High Resolution LC-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	Purity of commercially available highly-pure substances were determined by in-house qNMR purity assignment traceable to UME CRM 1301
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, MS ion
Extraction of analyte(s) of interest from matrix	✓	Solid-liquid extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Immuno Affinity Column (R-BIOPHARM . EASI EXTRACT AFLATOXIN RP70N)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	-
Analytical system	✓	High Resolution LC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	Isotope Dilution Mass Spectrometry (IDMS), five-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	-
Other	N/A	-

APPENDIX F: Summary of Participants' Analytical Information

The following Tables summarize the detailed information about the analytical procedures each participant provided in their "Analytical Information" worksheets. The presentation of the information in many entries has been consolidated and standardized.

The participant's measurement uncertainty statements are provided verbatim in Appendix G.

Table F.1. : Summary of Sample Size, Extraction, and Cleanup for CCQM-K138

Institute	Pre-treatment	Extraction Method	Sample Size (units)	Clean-up
BAM	Fifteen grams of the homogenised sample were weighed into a 120 mL polypropylene (PP) centrifugation tube	Fifteen grams of the homogenised sample were weighed into a 120 mL polypropylene (PP) centrifugation tube, followed by the addition of 1.5 g sodium chloride and 90 mL of a mixture of methanol/water (80:20, v/v), followed by the addition of 1.5 g sodium chloride and 90 mL of a mixture of methanol/water (80:20, v/v). The tube was closed and the mixture was shaken for 30 min at ambient temperature in a mechanical shaker (300 strokes/min).	15 g	The aqueous-methanolic layer was separated by centrifugation (ambient temperature, 10 min, 3000 rpm (1942 g)).
EXHM		11,4 g of the test material are mixed with water at 1:2 ratio to produce a slurry and is then spiked with labelled aflatoxins (13C17 B1, B2, G1 and G2) and left for 1 hour to equilibrate. 10 g of the slurry is mixed with 1 g NaCl and is then extracted with 60 mL MeOH:H2O 80:20 in a high sheer mixer for 3 min.	11.4 g (slurried with 23,6 g H2O)	Immunoaffinity column The extract is filtered and 16,5 g are mixed with 60 mL PBS buffer and pass through an IAC column. The column is washed with water and flushed with MeOH to collect the aflatoxins. The resultant solution is evaporated to dryness and redisolved in MeOH:H2O 2:1 and analysed in an LC-MS/MS system IA columns: Aflastar R (Rohmer Labs)

Institute	Pre-treatment	Extraction Method	Sample Size (units)	Clean-up
GLHK		Liquid/solid extraction by high speed homogenizer – 2 times extraction by water and followed by 3 times extraction by 80% methanol Volume of water used : 72 mL Volume of 80% methanol used 108 mL	6 g per analysis	Clean-up by immunoaffinity column
INTI		A test portion of 25g is extracted with MeOH-H2O (8+2). Extract is filtered, diluted with PBS and applied to an affinity column. Aflatoxins are removed from the affinity column with MeOH and are quantified by reversed-phase liquid chromatography with post-column derivatization involving bromination (Kobra cell) and determined by fluorescence detection.	25 g	Cleanup with immunoaffinity column. Chromatographic Separation with LC. Immunoaffinity Column Liquid Chromatography with Post-Column Derivatization (AOAC 999.07) Clean up method: Immunoaffinity column Aflatest WB VICAM - Elution solvent: MeOH
KEBS		Extraction by using a shaker and 80% Methanol SHAKING WITH 80% METHANOL Shake sample with extraction solvent for 40min, Filter using filter paper, 15mL filtrate mixed with 85mL PBS,	0.0	Immunoaffinity columns used 10mL (filtrate with PBS) loaded to IAC then washed with PBS. Elution with 2mL Methanol for HPLC

Institute	Pre-treatment	Extraction Method	Sample Size (units)	Clean-up
NIMT		Liquid-liquid extraction using 70:30 MeCN: water with 20 mL of extraction solvent: 10 grams sample 1. Weigh out 10 g of dried fig sample. An appropriate amount of each aflatoxin labeled solution and 1 g of NaCl were then added to the sample. 2. Add 20 mL of 70:30 MeOH:water to the mixture and mix vigorously for 60 mins 3. Centrifuge at 3000 rpm for 10 min, collect the supernatant and filter through 0.45 micron GMF. 4. Evaporate out organic consituent under N2 stream at 45 oC for 30 mins.	10 g	Immunoaffinity column (IAC) 5. Add 5 mL of 1XPBS (pH 7.4) and pass the mixture thorugh IAC column. 6. Wash the IAC with 5 mL water and elute with 2 portions of 2.5 mL acetonitrile. 7. Evaporate the eluate under N2 stream at 45 oC to dryness. 8. Reconsitute with 200 mL acetonitrile and filter with 0.2 micron PVDF before injecting onto HPLC
NMISA		Methanol: Water (80:20)saline solid-liquid extraction of the dried fig powder with shaking 60 min. Modified from the AOAC 999.07 method. In short, approximately 6-10 g of sample was weighed and extracted with 36 mL of extraction solvent and 10% NaCl (m/m sample). The samples were extracted for 1 hour by orbital shaking at approximately 200 rpm. A 12 mL aliquot of the extract was diluted into 60 mL of PBS,	6-10 g	Immunoaffinity clean-up (VICAM Aflatest) The full extract 72 mL was loaded onto a VICAM AFLAtest immunoaffinity clean up cartridge. Samples were eluted after washing, with 3 mL methanol. The eluate was dried down and resuspended in 300 µL of LC solvent.

Institute	Pre-treatment	Extraction Method	Sample Size (units)	Clean-up
VNIIM	10 g of sample was put into a 100-ml Erlenmeyer flask. the internal standards (13C17-aflatoxines B1. B2. G1. G2) and 40 mL of acetonitrile-water (84:16. v/v) were added.	SPE. After sonication for 30 minutes. the supernatant was filtered through a glass microfiber filter. Filtrate was purified by passing through the MycoSep 228 AflaPat cartridge at flow rate of 1 mL/min. The cleaned filtrate was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 1 mL of methanol-water (55:45 v/v). containing 10 mM ammonium acetate.	10 g	10 g of sample was put into a 100-ml Erlenmeyer flask. the internal standards (13C17-aflatoxines B1. B2. G1. G2) and 40 mL of acetonitrile-water (84:16. v/v) were added. After sonication for 30 minutes. the supernatant was filtered through a glass microfiber filter. Filtrate was purified by passing through the MycoSep 228 AflaPat cartridge at flow rate of 1 mL/min. The cleaned filtrate was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 1 mL of methanol-water (55:45 v/v). containing 10 mM ammonium acetate.
TUBITAKUME		Solid-liquid extraction 6 grams of sample were weighed into a 50 mL polypropylene centrifugation tube, and 100 uL IS stock solution added and weighed. Then, 0.6 g sodium chloride and 36 mL of extraction solvent (methanol:water 80:20 v/v) added. Tube wrapped with aluminum foil and vortex for 20 min at room temperature with Heidolph Multi Reax. Then centrifuge at 10000 rpm at 15 °C for 20 minutes. Extract was filtered through Macharey Nagel (product # 405012) glass fiber filter paper and 25 mL of filtered extract is diluted with 150 mL PBS buffer (pH 7.4, Sigma P4417).	6 g	Immuno affinity cleanup Diluted extract was transferred to reservoir on immuno affinity column (R-BIOPHARM EASI EXTRACT AFLATOXIN RP70N) and passed with application of vacuum, after extract was passed, column washed twice with 10 mL ultrapure water. Column dried for 5 seconds under vacuum. Aflatoxins eluted with 2 mL methanol to 4 mL amber vial by gravity. Concentrated under nitrogen stream till 0.5 mL remains. 1.5 mL ultrapure water added and vortex for 1 minute, if clear transfer to LC vial otherwise filter through 0.2 µm syringe filter. Analyze with Thermo Scientific Q Exactive Orbitrap HR-LC/MS.

Table F.2. Summary of Analytical Techniques for CCQM-K138

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
BAM	HPLC-SIDA-MS/MS	HPLC column: Agilent Zorbax Eclipse XDB C18. 2.1x100mm. 1.8µm.	Parameter Table CUR: 15.00 IS: 4000.00 TEM: 550.00 GS1: 70.00 GS2: 50.00 ihe: ON CAD: 4.00 EP 10.00 Dwell(msec): 50.00 Mobile phase: water and methanol (each 0.1% formic acid and 5mM ammonium formate; HPLC gradient). column temperature: 30°C. analysis time: 14.5 min. flow rate: 300 µL/min. injection volume: 5 µL	Scan Type: MRM (MRM). Scheduled MRM: No Polarity: Positive . Scan Mode: N/A. Ion Source: Turbo Spray. Resolution Q1: Unit. Resolution Q3: Unit. AFG1 quant: 329.000 → 243.100. DP 79.00. CE 39.00. CXP 14.00 1 AFG1 qual: 329.000 → 311.100. DP 79.00. CE 31.00. CXP 21.00 13C AFG1: 346.100 → 257.200. DP 94.00. CE 40.00. CXP 15.00 AFG2 quant: 331.000 → 285.100. DP 46.00. CE 38.00. CXP 18.00 AFG2 qual: 331.000 → 313.100. DP 46.00. CE 32.00. CXP 10.00 13C AFG2: 348.200 → 330.300. DP 94.00. CE 35.00. CXP 23.00 AFB1 quant: 313.000 → 285.200. DP 86.00. CE 33.0. CXP 18.00 AFB1 qual: 313.000 → 241.200. DP 86.00. CE 52.00. CXP 13.00 13C AFB1: 330.200 → 301.200. DP 91.00. CE 35.00. CXP 19.00 AFB2 quant: 315.000 → 287.100. DP 66.00. CE 37.00. CXP 18.00 AFB2 qual: 315.000 → 259.100. DP 66.00. CE 43.00. CXP 15.00 13C AFB2: 332.000 → 332.100. DP 91.00. CE 39.00. CXP 18.00

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
EXHM	ID-LC-MS/MS	Waters XTerra MS 15 mm. 2.1 mm. 3 µm	HESI - multiple reaction monitoring Capillary Temp: 270, Vaporizer Temp: 350, Sheath Gas Pressure: 40.0, Ion Sweep Gas Pressure: 0.0, Aux Gas Flow: 10.0, Spray Voltage: + 4000.0 Mobile phase: Water (A) - MeOH (B) gradient: 0 min - 90A/10B. 4 min90A/10B. 12 min 30A/70B. 16 min 10A/90B. 20 min 10A/90B. 21 min 90A/10B. 25 min 90A/10B flow rate: 150 mL. injection vol. 20 mL	AfB1 (313.1 to 285Q. 241q). AflaB2 (315.1 to 243. 259Q. 287q). AflaG1 (329.1 to 200q. 215. 243Q). AflaG2 (331.1 to 201. 217. 245Q. 257q. 275. 313). 13C-AfB1 (330.1 to 255. 301). 13CAflaB2 (332 to 259. 303). 13CAflaG1 (346 to 212. 317). 13CAflaG2 (348 to 259. 313)

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
GLHK	LC-MS/MS	ACQUITY UPLC C18 (2.1 x 100 mm. 1.7 µm)	Operation mode : ESI positive ionization Source temperature : 450 °C Ion spary voltage : 5500 V Mobile phase A : 10mM ammonium formate. 0.1% formic acid. 5% MeOH in water Mobile phase B : methanol Gradient program : t = 0min. 95%A; t = 1min. 60%A; t =7. 55%A; t = 7.5-10.5min. 5%A; t = 11-15min. 95%A Flow rate : 0.25 mL/min Analysis time : 15 min Injection volume : 20 µL Column temperature 35 °C	MRM scanning Operation mode : ESI positive ionization Source temperature : 450 °C Ion spray voltage : 5500 V
INTI	Inmunaffinity columns, electrochemical derivatization (Kobra Cell) and LC with fluorescence detection	Reversed Phase Column ODS 4.6 mm x 15 cm, 5 um.	Mobile Phase: H20+MeOH (6+4) + 216.4 mg KBr/L +159.1 ul (HNO3 4N)/L. Fluorescence detector wavelengths 360 nm excitation filter and 420 nm emission filter. Column Temperature: 40°C. Column Pressure 61 bar. Analysis time 20 min. Flow rate 1 ml/min. Injection Volume 100 ul.	
KEBS	HPLC- FL DETECTION	C18 150 mm 5u column	MP- Water:Methanol:CAN (5:4:1). Detector FL Ex365 Em435. Temp 30. Flow rate 1ml/min. Analysis time 9 min. 10uL injection	

<table>
<tr><th>Institute</th><th>Analytical Technique</th><th>Chromatographic Column</th><th>Chromatographic and Mass Spectrometry Conditions</th><th>ion/MRM monitored</th></tr>
<tr><td>NIMT</td><td>LC-MS/MS IDMS</td><td>Luna C18 4.6x150 mm 5 mm 100 Å</td><td>Detection by MS/MS:Positive ESI with SRM mode

Chromatographic conditions: MP: MeCN:H2O with 20 mM Formic acid (42:58) Flow rate: 0.5 mL/min
Injection vol: 20 mL
Column temp: 40 °C</td><td>AFB1: 313.1>241. 313.1>268 labeled AFB1: 330.1>301.1. 330.1>255.1
AFB2: 315.1>259.05. 315.1>287.1 labeled AFB2: 332.2>303.2. 332.2>273.1
AFG1: 329.1>242.95. 329.1>200.1 labeled AFG1: 346.1>257.1. 346.1>212.1
AFG2; 331.15>245. 331.15>275 labeled AFG2: 348.1>330.1. 348.1>259.1</td></tr>
<tr><td>NIMSA</td><td>UPLC-MS/MS</td><td>Acquity UPLC BEH C18 1.7 µm. 2.1 x 100 mm column (40°C)</td><td>The cone voltage was set at 2 V with a collision energy for the various transitions range from 24 to 40 eV. The capillary voltage was set at 2.5 kV and the desolvation temperature at 550°C. The cone gas flow was set to 150 L/h whilst the desolvation gas flow was 800 L/h.

Mobile phase 5 mM ammonium formate aqueous and methanol solvents at a flow rate of 0.35 mL/min and the total runtime was 7.5 min. The maximum pressure reached during a run is approximately 11500 psi.</td><td>The MS/MS analysis was performed on a WatersTQS triple quadrupole instrument
The quantifier transitions for each of the toxins was:
<table><tr><th></th><th>Parent</th><th>Fragment</th></tr><tr><td>AFB1</td><td>313.16</td><td>284.918</td></tr><tr><td>AFB2</td><td>315.16</td><td>287.047</td></tr><tr><td>AFG1</td><td>329.096</td><td>242.988</td></tr><tr><td>AFG2</td><td>331.16</td><td>244.973</td></tr><tr><td>13C AFB1</td><td>330.138</td><td>300.932</td></tr><tr><td>13C AFB2</td><td>332.202</td><td>303.053</td></tr><tr><td>13C AFG1</td><td>346.138</td><td>256.978</td></tr><tr><td>13C AFG2</td><td>348.138</td><td>259.049</td></tr></table>.</td></tr>
<tr><td>VNIIM</td><td>LC-MS/MS IDMS</td><td>Hydroshere C18 100mm x 4,6 mm, 3 µm;</td><td>ESI(+)
Mobile phase: A - ammonium acetete 10mmol/L (45%).
B - methanol (55%); isocratic eluation; flow rate 0.8 ml/min; column temperature 30°C; injection volume 5 µl</td><td>ESI(+); MRM: aflatoxin B1 (313 → 241). aflatoxin B2 (315 → 259). aflatoxin G1 (329 → 243). aflatoxin G2 (331 → 245). 13C17-aflatoxin B1 (330 → 255). 13C17-aflatoxin B2 (332 → 273). 13C17-aflatoxin G1 (346 → 257).13C17-aflatoxin G2 (348 → 259).</td></tr>
</table>

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
TUBITAK UME	HR-LC-MS IDMS		MS Resolution: 70000 HESI Positive Capillary Temperature 280 °C Aux gas heater temp. 250 °C Sheath gas flow rate: 45 Aux gas flow rate: 10 Spray voltage (kV): 3.60 Scan range: 100 - 1000 m/z mobile phase: A: 95 % water 5 % MeOH 5 mM Ammonium Acetate 0.1 % B: MeOH column temperature: 40°C. Autosampler 4 °C injection volume: 10 µL Ret(min) Flow (µL/min) % A % B 00 0.3 95 5 06 0.3 50 50 10 0.3 5 95 15 0.3 5 95 15.1 0.3 95 5 18 0.3 95 5	B1 : 313.0700 B1-13C17 : 330.1270 B2 : 315.0860 B2-13C17 : 332.1430 G1 : 329.0650 G1-13C17 : 346.1220 G2 : 331.0810 G2-13C17 : 348.1370

Table F.3. Summary of Calibrants and Standards for CCQM-K138

Institute	Type of Calibration	Calibrants	Internal Standards
BAM	Six point internl standard calibration (SIDA), IDMS, linear regression	Commercial standards (Biopure, RomerLabs), gravimetric sample preparation Aflatoxin B1 in Acetonitril (2.01µg/mL +/- 0.03 µg/mL; Biopure) B1: 16192B Aflatoxin B2 in Acetonitril (0.502µg/mL +/- 0.008 µg/mL; Biopure) B2: L15483A Aflatoxin G1 in Acetonitril (2.01µg/mL +/- 0.03 µg/mL; Biopure) G1: L15331C Aflatoxin G2 in Acetonitril (0.500µg/mL +/- 0.008 µg/mL; Biopure) G2: L15391A	Alfa -Mix 13C17-B1: I15383M 13C17-B2: I15383M 13C17-G1: I15383M 13C17-G2: I15383M
EXHM	Exact matching matrix matched standards ID-LC-MS/MS	IRMM ERM–AC057 IRMM ERM – AC058 IRMM ERM – AC059 IRMM ERM – AC060	C13 labelled aflatoxin solutions were purchased from LGC (B1) and Romer Labs (B2. G1. G2)

Institute	Type of Calibration	Calibrants	Internal Standards
GLHK	3 - 5 calibration points Quantification mode used - Isotope Dilution Mass Spectrometry Calibration mode used – Standard addition	Aflatoxin B1 : IRMM ERM – AC057 Aflatoxin B2 : IRMM ERM – AC058 Aflatoxin G1 : IRMM ERM – AC059 Aflatoxin G2 : IRMM ERM – AC060	[$^{13}C_{17}$] Aflatoxin B_1 from LGC [$^{13}C_{17}$] Aflatoxin B_2 from LGC [$^{13}C_{17}$] Aflatoxin G_1 from LGC [$^{13}C_{17}$] Aflatoxin G_2 from LGC
INTI	External standard, 5 points calibration curve Spectrophotometric method (AOAC 971.22)	Fluka AG, Aflatoxins B1, B2, G1 and G2 from Aspergillus Flavus Aflatoxin B1 Cat. Code: 5032 Batch 2216541280. Aflatoxin B2 Cat. Code: 5033 Batch 202621578. Aflatoxin G1 Cat. Code:5035 Batch 219939181. Aflatoxin G2 Cat. Code: 5036 Batch 219940181.	-
KEBS	External Calibration	AFB1 Source FERMENTEK Lot# AF017 AFB2 Source TRILOGY ANALYTICAL LABORATORY Lot# 141104-070 AFG1 Source TRILOGY ANALYTICAL LABORATORY Lot# 150305-070 AFG2 Source TRILOGY	-

Institute	Type of Calibration	Calibrants	Internal Standards
		ANALYTICAL LABORATORY Lot# 150309-070	
NIMT	6-pt calibration with labeled internal standard, IDMS	IRMM ERM–AC057 IRMM ERM – AC058 IRMM ERM – AC059 IRMM ERM – AC060	labeled AFB1 labeled AFB2 labeled AFG1 labeled AFG2
NIMSA	Double IDMS, standard addition. External standard 9-point std addition; 6-point external calibration; 3 brackets dIDMS.	IRMM ERM–AC057, ILM010 Lot I15231A IRMM ERM – AC058, ILM011 Lot I15345B IRMM ERM – AC059, ILM012 Lot I15345A IRMM ERM – AC060, ILM013 Lot I15232G	BIOPURE 13C Afla B1 BIOPURE 13C Afla B2 BIOPURE 13C Afla G1 BIOPURE 13C Afla G2
VNIIM	Single point, IDMS	Commercial standards Aflatoxin B1 in acetonitrile. Biopure Aflatoxin B2 in acetonitrile. Biopure Aflatoxin G1 in acetonitrile. Biopure Aflatoxin G2 in acetonitrile.	13C17-aflatoxin B1 solution in acetonitrile (cat. № ILM010). 13C17-aflatoxin B2 solution in acetonitrile (cat. № ILM011). 13C17-aflatoxin G1 solution in acetonitrile (cat. № ILM012) 13C17-aflatoxin G2 solution in acetonitrile (cat. № ILM013) were obtained from Biopure.

Institute	Type of Calibration	Calibrants	Internal Standards
		Biopure	
TUBITAK UME	Five point internal standard calibration, IDMS	Commercial standards purity determined by QNMR traceable to UME CRM 1301, gravimetric sample preparation AFB1 SIGMA A6636 AFB2 SIGMA A9887 AFG1 SIGMA A0138 AFG2 SIGMA A0263	AFB1-13C17 SIGMA 32764l AFB2-13C17 SIGMA 32771 AFG1-13C17 SIGMA 32772 AFG2-13C17 SIGMA 32777

Table F.4. Assessment and Verification Methods for CCQM-K138

Institute	Purity Assessment	Result Verification
BAM	The purity of the used certified calibration standards on three ways: - Purities of calibration standards were independently confirmed by LC-MS measurements (scan mode; ESI+/-). The specified aflatoxin contents of the used certified standard solutions were cross checked by certified standards of different lot numbers (same provider) Additional cross check using certified standard solutions of a second provider.	-
EXHM	The solid aflatoxins used by EXHM have been characterized for their purity using the mass balance approach and qNMR. The concentration of the solutions prepared has been assigned against the IRMM CRM solutions (ERM AC 057, 058, 059, 060) using IDMS experiments, and this is the reason why we attribute traceability to IRMM. The actual values were: AFB1=96.13±3.18%, AFB2=93.32±3.13%, AFG1=98.60±3.35%, AFG2=94.02±3.12%.	-
GLHK	-	-

Institute	Purity Assessment	Result Verification
INTI	One solution of each aflatoxin was prepared to obtain 4 stock solutions of 8-10 ug/ml in acetonitrile. These solutions were verified using an Spectrophotometric method (AOAC 971.22). After the measurement of the stock solution at 350nm, it was adjusted the purity of each calibration solution. The assignment of purity was determinated following the next equation: % purity = ccstandard stock x 10ml x 5000ul x100 50 ul x 1000 ug/mg x 10 mg	We do not use any verification. Verification is not necessary due specificity of cleanup separation used in the method.
KEBS	-	-
NIMT	-	-
NIMSA	-	-
VNIIM	-	-

Institute	Purity Assessment	Result Verification
TUBITAK UME	Purity of commercially available highly-pure substances were determined by in-house qNMR purity assignment traceable to UME CRM 1301	-

Table F.5. Additional Comments for CCQM-K138

Institute	Additional Comments
BAM	Remarks: - LOD/LOQ (as mass fraction): 0.083 µg/kg / 0.328 µg/kg for AFB_1, 0.033 µg/kg / 0.128 µg/kg for AFB_2, 0.136 µg/kg / 0.539 µg/kg for AFG_1, 0.016 µg/kg / 0.064 µg/kg for AFG_2 Information of quality control sample: None
EXHM	Remarks: -C13 labelled aflatoxin solutions were purchased from LGC (B_1) and Romer Labs (B_2, G_1,G_2) -The product ions 259 and 245 were used to quantify Afla B2 and Afla G2 respectively, due to pronunced matrix interference for the more abundant ions. -IA columns: Aflastar R (Rohmer Labs) LOD/LOQ (as mass fraction): 5/15 ng/g Information of quality control sample: FAPAS T04280QC
GLHK	Remarks : Concentration of calibrants in standard addition does not include the concentration of AFs from sample LOD/LOQ (as mass fraction): LOQ of analyte calibrated by standard addition is regarded as the sample concentration in mass fraction Information of quality control sample: IRMM ERM – BE375 Compound Feedingstuff

INTI	Remarks: Preparation of standards: 1) Preparation of Calibrant: To container of 10 mg of each dry aflatoxin was added a volume of 5 ml of Toluene : Acetonitrile (9+1). Final concentration aprox. 2 mg/ml. 2) Preparation of stock solution: From calibrant solution (50ul) were prepared individuals stock solution in acetonitrile of each aflatoxin. Final concentration (10 ml) aprox. 8-10 ug/ml. 3) Working solution: The working solutions were prepared mixed the four toxins from stock solutions. The solutions were prepared in four levels: 0.075 ng/ml B1, B2, G1 and G2 - 0.375 ng/ml B1, B2, G1 and G2- 1.25 ng/ml B1, B2, G1and G2 - 2.5 ng/ml B1, B2, G1 and G2. The accuracy of the method was determinated making a recovery test in-house material. The results obtained were the following: AfB1: 112%, AfB2 88% AfG1 110%, AfG2 93%. The repetibility was determinated analyzing each sample four times in the same day as individual replicates. LOD/LOQ (as mass fraction): 0.1 ng/g / 0.3 ng/g Information of quality control sample: Recovery test using in-house material (Recovery values 88%-112%)
KEBS	Remarks: N/A LOD/LOQ (as mass fraction): N/A Information of quality control sample: CRM-ERMBE375
NIMT	Remarks:- LOD/LOQ (as mass fraction): 0.3/0.8 ng/g for B_1, 0.06/0.15 ng/g for B_2, 0.14/0.4 ng/g for G_1, 0.03/0.1 ng/g for G_2 Information of quality control sample: Spiked blank

NIMSA	Remarks: -The solvent proportions were maintained when extracting increased masses of sample. -Recovery on QC >90% -Matrix enhancement effects were observed (compensated for by the isotope) and there was limited stability of the low concentration calibrant solutions for G1 and G2. The homogeneity of the sample appears to be a significant contributor to the variability as multiple aliquots from a single extract yielded very similar results, suggesting that the large variability between repeat analyses is not as a result of the clean-up and analytical method. Initial tests were run using HPLC-FLD which confirmed data obtained using LC- MS/MS. FAPAS fig slurry was used as QC, recoveries >90% achieved. LOD/LOQ (as mass fraction): 0.14/ 0.46 ng/g for AB_1 0.027/ 0.090 ng/g for AB_2 0.074/ 0.25 ng/g for $AG_1$0.022/ 0.074 ng/g for AG_2 Information of quality control sample: FAPAS T04258 Fig Slurry 1.72 µg/kg (0.96 - 2.48) for AFB_1 FAPAS T04258 Fig Slurry 1.30 µg/kg (0.73 - 1.87) for AFB_2 FAPAS T04258 Fig Slurry 0.94 µg/kg (0.52 - 1.35) for AFG_1 FAPAS T04258 Fig Slurry 0.88 µg/kg (0.49 - 1.27) for AFG_2
VNIIM	Remarks: Internal standards: 13C17-aflatoxin B1 solution in acetonitrile (cat. № ILM010), 13C17-aflatoxin B2 solution in acetonitrile (cat. № ILM011), 13C17-aflatoxin G1 solution in acetonitrile (cat. № ILM012) and 13C17-aflatoxin G2 solution in acetonitrile (cat. № ILM013) were obtained from Biopure. LOD/LOQ (as mass fraction): N/A Information of quality control sample: Sample of dried fig with addition of AFB1, AFB2, AFG1, AFG2
TUBITAK UME	Remarks: - LOD/LOQ (as mass fraction): 0.029 µg/kg / 0.096 µg/kg for AB_1 , 0.003 µg/kg / 0.009 µg/kg AB_2 0.008 µg/kg / 0.023µg/kg for AG_1 , 0.001 µg/kg / 0.002 µg/kg AG_2 Information of quality control sample: None

APPENDIX G: Summary of Participants' Uncertainty Estimation Approaches

The following are text excerpts and/or pictures of the uncertainty-related information provided by the participants in the reporting form. Information is grouped by participant and presented in alphabetized acronym order.

Uncertainty Information from BAM

w_sample= ((r_area - i_cal)/sl_cal) · m_is/m_sample
w_sample: mass fraction of aflatoxin in sample
r_area: area ratio native compound/internal standard
i_cal: intercept of calibration line
sl_cal: slope of calibration line
m_is: mass of internal standard added to sample
m_sample: mass sample

Uncertainty estimation was performed. using the following equation:

U_(95%)=k · √((s/m)^2+ (u(c_cal)/c_cal)^2+ (u_(x_pred)/x_pred)^2)

U_(95%): expanded uncertainty 95% confidence
k: coverage factor
m: mean
s: standard deviation of the mean
u_(c_cal): uncertainty of the standard substances
u_(x_pred): uncertainty of the calibration

where u_(x_pred) was calculated according to EURACHEM CITAC Guide:

var(x_pred)= S^2/(b_1^2) · (1/p+ 1/n + (x_pred- $\bar{x}$)^2/((∑(x_i^2)-(∑x_i)^2/n))) ;
S^2= (∑w_i (y_i-y_fi)^2))/((n-2))

(y_i-y_fi): residual for the ith point
n: number of data points in the calibration
b_1 : calculated best fit gradient
p: number of measurements
x_i. y_i: data points
x_pred: estimated concentration
$\bar{x}$: mean

Estimation of standard measurement uncertainties:

method precision: standard deviation of the mean (n = 6)
standard substances: based on given uncertainties of the standards
calibration: uncertainties from linear least squares calibration according to EURACHEM CITAC Guide

Uncertainty estimation for u_c;sumAfla was performed. using the following equation:

u_c;sumAfla=√((u_c;AFB1)^2 + (u_c;AFB2)^2 + (u_c;AFG1)^2 + (u_c;AFG2)^2)

u_c;AF: combined uncertainty of the respective aflatoxin

Uncertainty Information from EXHM/GCSL-EIM

The measurement equation is:

$$w_{M.S} = w_{M.C}\,\frac{100}{Rec} \times \frac{m_{is.S}}{F \times m_{M.S}} \times \frac{m_{M.C}}{m_{is.C}} \times \frac{R_S}{R_C}$$

where $w_{M.S}$ = aflatoxin mass fraction in the sample. ($\mu g/kg$)
$w_{M.C}$ = aflatoxin mass fraction in the calibration solution. ($\mu g/kg$)
F = sample fraction in slurry (g/g)
Rec = recovery (%). assessed against other independent methods
$m_{is.S}$ = mass of internal standard solution added to sample blend. (g)
$m_{M.S}$ = mass of slurry in sample blend. (g)
$m_{M.C}$ = mass of the calibration solution added to calibration blend. (g)
$m_{is.C}$ = mass of internal standard solution added to calibration blend. (g)
R_S = measured peak area ratio of the selected ions in the sample blend
R_C = measured peak area ratio of the selected ions in the calibration blend

The equation used to estimate standard uncertainty is:

$$u(w_{BS}) = \sqrt{\left({}^{s_R}/_{\sqrt{n}}\right)^2 + \sum\left(C_j u(m_i)\right)^2 + \sum\left(C_j u(R_i)\right)^2 + \left(C_j u(w_{MC})\right)^2 + + \left(C_j u(R)\right)^2 + \left(C_j u(F)\right)^2}$$

where s_R is the standard deviation under reproducibility conditions. n the number of determinations and C_j the sensitivity coefficients associated with each uncertainty component. The uncertainty of the peak area ratios was considered to have been included in the estimation of method precision.

Uncertainty estimation was carried out according to JCGM 100: 2008. The standard uncertainties were combined as the sum of the squares of the product of the sensitivity coefficient (obtained by partial differentiation of the measurement equation) and standard uncertainty to give the square of the combined uncertainty. The square root of this value was multiplied by a coverage factor (95% confidence interval) from the t-distribution at the total effective degrees of freedom obtained from the Welch-Satterthwaite equation to give the expanded uncertainty.

The uncertainty budgets for the four aflatoxins are shown in the pages that follow.

Aflatoxin B1

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	5,994	1,0000	0,066	0,0110	0,0659	0,0043
mass fraction of AFLA B1 in the calibration solution, (ng/g)	400,81	0,0150	5,23	0,0130	0,0782	0,0061
slurry concentration, (g K138 sample/g slurry)	0,3257	-0,2071	0,0002	0,0005	0,0000	0,0000
recovery (%)	100,00	-0,0599	1,130	0,0113	-0,0677	0,0046
mass of $^{13}C_{17}$-AFLA B1 solution added to sample blend, (g)	0,07394	81,0616	0,00007	0,0009	0,0057	0,0000
mass of slurry in sample blend, (g)	10,0000	-0,5994	0,00032	0,0000	-0,0002	0,0000
mass of AFLA B1 solution added to calibration blend, (g)	0,04723	13,4633	0,00003	0,0006	0,0004	0,0000
mass of $^{13}C_{17}$-AFLA B1 solution added to calibration blend, (g)	0,07366	-81,3697	0,00003	0,0004	-0,0024	0,0000
measured peak area ratio of the selected ions in the sample blend	2,0876	2,8711	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	2,0320	-2,9497	estimation of method precision			

result (ng/g)	5,994
combined standard uncertainty (ng/g)	0,123
relative standard uncertainty (%)	2,05
effective degrees of freedom	37,3
coverage factor	2,03
expanded uncertainty (ng/g)	0,249

Aflatoxin B2

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	0,871	1,0000	0,018	0,0209	0,0182	0,0003
mass fraction of AFLA B2 in the calibration solution, (ng/g)	45,44	0,0192	0,42	0,0092	0,0081	0,0001
slurry concentration, (g K138 sample/g slurry)	0,3257	-0,0301	0,0002	0,0005	0,0000	0,0000
recovery (%)	100,00	-0,0087	1,130	0,0113	-0,0098	0,0001
mass of $^{13}C_{17}$-AFLA B2 solution added to sample blend, (g)	0,05448	15,9869	0,00007	0,0013	0,0011	0,0000
mass of slurry in sample blend, (g)	10,0000	-0,0871	0,00032	0,0000	0,0000	0,0000
mass of AFLA B2 solution added to calibration blend, (g)	0,06069	1,5225	0,00003	0,0005	0,0000	0,0000
mass of $^{13}C_{17}$-AFLA B2 solution added to calibration blend, (g)	0,05554	-15,6818	0,00003	0,0005	-0,0005	0,0000
measured peak area ratio of the selected ions in the sample blend	0,0581	14,9908	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	0,0554	-15,7214	estimation of method precision			

result (ng/g)	0,871
combined standard uncertainty (ng/g)	0,022
relative standard uncertainty (%)	2,55
effective degrees of freedom	17,3
coverage factor	2,11
expanded uncertainty (ng/g)	0,047

Aflatoxin G1

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	2,093	1,0000	0,042	0,0202	0,0423	0,0018
mass fraction of AFLA G1 in the calibration solution, (ng/g)	126,01	0,0166	2,18	0,0173	0,0362	0,0013
slurry concentration, (g K138 sample/g slurry)	0,3257	-0,0723	0,0002	0,0005	0,0000	0,0000
recovery (%)	100,00	-0,0209	1,130	0,0113	-0,0237	0,0006
mass of $^{13}C_{17}$-AFLA G1 solution added to sample blend, (g)	0,03951	52,9765	0,00007	0,0018	0,0037	0,0000
mass of slurry in sample blend, (g)	10,0000	-0,2093	0,00032	0,0000	-0,0001	0,0000
mass of AFLA G1 solution added to calibration blend, (g)	0,05117	4,3396	0,00003	0,0006	0,0001	0,0000
mass of $^{13}C_{17}$-AFLA G1 solution added to calibration blend, (g)	0,03917	-53,4363	0,00003	0,0008	-0,0016	0,0000
measured peak area ratio of the selected ions in the sample blend	1,3260	1,5785	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	1,2650	-1,6546	estimation of method precision			

result (ng/g)	2,093
combined standard uncertainty (ng/g)	0,061
relative standard uncertainty (%)	2,90
effective degrees of freedom	23,6
coverage factor	2,07
expanded uncertainty (ng/g)	0,125

Aflatoxin G2

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	0,264	1,0000	0,009	0,0345	0,0091	0,0001
mass fraction of AFLA G2 in the calibration solution, (ng/g)	12,63	0,0209	0,14	0,0111	0,0029	0,0000
slurry concentration, (g K138 sample/g slurry)	0,3257	-0,0091	0,0002	0,0005	0,0000	0,0000
recovery (%)	100,00	-0,0026	1,130	0,0113	-0,0030	0,0000
mass of $^{13}C_{17}$-AFLA G2 solution added to sample blend, (g)	0,05940	4,4374	0,00007	0,0012	0,0003	0,0000
mass of slurry in sample blend, (g)	10,0000	-0,0264	0,00032	0,0000	0,0000	0,0000
mass of AFLA G2 solution added to calibration blend, (g)	0,06497	0,4304	0,00003	0,0005	0,0000	0,0000
mass of $^{13}C_{17}$-AFLA G2 solution added to calibration blend, (g)	0,05903	-4,4652	0,00003	0,0005	-0,0001	0,0000
measured peak area ratio of the selected ions in the sample blend	0,8796	0,2997	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	0,8460	-0,3116	estimation of method precision			

result (ng/g)	0,264
combined standard uncertainty (ng/g)	0,010
relative standard uncertainty (%)	3,80
effective degrees of freedom	11,6
coverage factor	2,20
expanded uncertainty (ng/g)	0,022

Uncertainty Information from GLHK

The mass fraction of aflatoxins (AFB_1.AFB_2. AFG_1. AFG_2) were quantified by isotope dilution mass spectrometry (IDMS). Standard addition was employed for calibration in this comparison.

Measurement equations used :

1) Linear equation for standard addition

$$y_i = a_1 \cdot x_i + a_0 \quad \text{where} \quad y_i = R_i \frac{m_{y,i}}{m_{x,i}} \ , \ x_i = \frac{m_{z,i}}{m_{x,i}}$$

where

i - i^{th} solution of standard addition

R_i - peak area of analyte/ peak area of IS of the i^{th} solution of standard addition

$m_{x.i}$ - mass of sample x in the ith solution of standard addition

$m_{y.i}$ - mass of IS solution y added to the i^{th} solution of standard addition

$m_{z.i}$ - mass of added standard z in the i^{th} solution of standard addition

a_0 - y-intercept of the linear fit function of standard addition calibration curve

a_1 - slope of the linear fit function of standard addition calibration curve

2) Equation for mass fraction calculation

$$w_x = \frac{a_0}{a_1} \cdot w_z$$

where

w_x - mass fraction of the analyte in sample x

w_z - mass fraction of the analyte in standard z

Individual uncertainty contributions

1) Uncertainty in mass fraction. w_x calibrated by standard addition

$$\left(\frac{u(w_x)}{w_x}\right)^2 = \left(\frac{u(w_z)}{w_z}\right)^2 + \frac{S^2}{a_0^2} \cdot \left[\frac{1}{n} + \frac{\left(\frac{w_x}{w_z} + \bar{x}\right)^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}\right]$$

where

$$S^2 = \frac{\sum_{i=1}^{n}[y_i - (a_0 + a_1 \cdot x_i)]^2}{n-2}$$

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

2) Weighing - mass of sample. mass of standard added and mass of IS added
- estimated by combining uncertainty in weighing. including uncertainty from analytical balances

3) Precision
- estimated by the variation in response factors from repeated measurement of samples

4) Recovery
- estimated by recovery of blank sample spikes calibrated by standard addition method

5) Uncertainty of mean value
- estimated from the deviation of mass fractions from 2 different sample units

Combining individiual uncertainties

Take AFB_1 as an example. combining individual uncertainties for each of the sample unit:

Sample 145		C_{B1} (ng/g)	Weighing	Recovery	Precision
	Value (x_i)	5.6345	1	1	1
	$u(x_i)$	2.1958E-01	3.2150E-04	2.8355E-03	6.5841E-02
C_{B1} (ng/g)	5.6345	5.8541	5.6345	5.6345	5.6345
Weighing	1	1.0000	1.0003	1.0000	1.0000
Recovery	1	1.0000	1.0000	1.0028	1.0000
Precision	1	1.0000	1.0000	1.0000	1.0658
C_{B1} (ng/g)	5.6345	5.8541	5.6364	5.6505	6.0055
$u(y,x_i)$		2.1958E-01	1.8115E-03	1.5977E-02	3.7099E-01
$u(y,x_i)^2$	0.1861	4.8213E-02	3.2816E-06	2.5526E-04	1.3763E-01
$u(C_{B1})$, (ng/g)	0.4314				

Sample 340		C_{B1} (ng/g)	Weighing	Recovery	Precision
	Value (x_i)	5.8681	1	1	1
	$u(x_i)$	2.8325E-01	3.1265E-04	2.8355E-03	7.8778E-02
C_{B1} (ng/g)	5.8681	6.1514	5.8681	5.8681	5.8681
Weighing	1	1.0000	1.0003	1.0000	1.0000
Recovery	1	1.0000	1.0000	1.0028	1.0000
Precision	1	1.0000	1.0000	1.0000	1.0788
C_{B1} (ng/g)	5.8681	6.1514	5.8700	5.8848	6.3304
$u(y,x_i)$		2.8325E-01	1.8347E-03	1.6639E-02	4.6228E-01
$u(y,x_i)^2$	0.2942	8.0233E-02	3.3660E-06	2.7686E-04	2.1370E-01
$u(C_{B1})$, (ng/g)	0.5424				

Include also the standard uncertainty of the mean value and calculate the overall expanded uncertainty. k = 2:

AFB_1 Mean Value		
Mean AFB_1 =	5.7513	ng/g
u(between-bottle deviation) =	0.1168	ng/g
u (AFB_1) =	0.5443	ng/g
U (AFB_1) =	1.0886	ng/g

Uncertainty Information from INTI

Measurement Equation of each analyte: CCinter = Interception of calibration curve from repetibility of each sample
V1 = 5 ml with volumetric flask
V2 = 20 ml with Volumetric pipette
V3 = 30 ml with Graduated cylinder
V4 = 9 ml con Adjustable pipette
V5 = 100 ml with Graduated Cylinder
V6 = 30 ml + 9 ml (Graduated Cylinder + Adjustable pipette)
m = mass with laboratory balance

Measurement equation to calculate uncertainty:
ΔCCinter afx= Measurement uncertainty
ΔCCinter = Interception of calibration curve from repetibility of each sample - 0.0751ng/ml; 0.0284ng/ml; 0.0160ng/ml; 0.0094ng/ml (Afx B1; Afx B2; Afx G1; Afx G2)
ΔV1 = 0.014 ml with volumetric flask (internal calibration)
ΔV2 = 0.0176 ml with Volumetric pipette (internal calibration)
ΔV3 = 0.151ml with Graduated cylinder (internal calibration)
ΔV4 = 0.005 ml con Adjustable pipette (internal calibration)
ΔV5 = 0.0939 ml with Graduated Cylinder (internal calibration)
ΔV6 = 0.1515 ml (Graduated Cylinder + Adjustable pipette) (internal calibration)
Δm = 0.01 g mass with laboratory balance (internal calibration)

Uncertainty Information from NIMT

wx= Mass fraction of aflatoxin (ng/g) in test sample

w0 = Mass fraction ratio (between unlabeled/labeled) obtained from the calibration curve (ng/ng)

wy(x) = Mass fraction of aflatoxin internal standard added to the sample. ng/g

my(x) = Mass of internal standard spiked into the sample (g)

mx= Mass of sample (g)

R = Recovery factor

$$w_x = w_o w_{Y(X)} \frac{m_{y(x)}}{m_x} \frac{1}{R}$$

u(my). u(mx) = standard uncertainties due to weighing estimated from bias of balance
u(w0)= standard uncertainty of the mass fraction ratio (between unlabeled/labeled) obtained from the calibration curve (ng/ng) estimated from the regression
u(Fcal) = standard uncertainty of mid concentration calibration standard estimated from bias and random effects (type B and type A)
u(FE) = standard uncertainty of extraction

u(FP) = standard uncertainty of method precision

u(R) = standard uncertainty of recovery

$$\frac{u(w_x)}{w_x} = \sqrt{\left(\frac{u(m_y)}{m_y}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(w_0)}{w_0}\right)^2 + \left(\frac{u(F_{cal})}{F_{cal}}\right)^2 + \left(\frac{u(F_E)}{F_E}\right)^2 + \left(\frac{u(F_P)}{F_P}\right)^2 + \left(\frac{u(R)}{R}\right)^2}$$

Measurement equation for determining ($\bar{Y}$), the mass fraction of Aflatoxin in fig test portion:

$$\bar{Y} = \frac{(Y_{dIDMS} + Y_{std\ add} + Y_{ext})}{3} \left(\frac{ng}{g}\right)$$

Where:

$$Y_{dIDMS} = W_z \times \frac{m_z}{m_{yc}} \times \frac{m_y}{m_x} \times \frac{R'_B}{R'_{BC}}$$

Y_{dIDMS}	Mass fraction of Aflatoxin in fig test portion (ng/g) obtained using bracketing double-isotope dilution
W_z	Mass fraction of aflatoxin in calibration CRM (g)
m_z	Mass of CRM added to calibration blend (g)
m_{yc}	Mass of isotope added to calibration blend (g)
m_y	Mass of isotope added to sample blend (g)
m_x	Mass of test sample (g)
R'_B	Peak area ration of analyte/isotope in sample blend
R'_{BC}	Peak area ration of analyte/isotope in calibration blend

$$Y_{std\ add} = X_{intercept} \times D \times Rec$$

Similarly:

$$Y_{ext} = X_{intercept} \times D \times Rec$$

Where:

$$X_{intercept} = \frac{c}{m}$$

Derived from:

$$y = mx + c$$

Where through linear regression of the calibration data:

$Y_{std\ add}$	Mass fraction of Aflatoxin in fig test portion (ng/g) obtained using standard addition
Y_{ext}	Mass fraction of Aflatoxin in fig test portion (ng/g) obtained using external calibration
$X_{intercept}$	Mass fraction of aflatoxin in calibration CRM (g)
y	Peak area of analyte
m	Slope
x	Mass fraction (ng/g) of calibration CRM added
c	y-intercept
D	Dilution factor
Rec	Recovery factor applied, determined from isotope recovery standard

The final uncertainty estimate for each toxin is calculated by combining the uncertainty of each result according to the following equation:

$$U_{95}(\bar{Y}) = 2 \times \sqrt{\frac{\left(\sum_{j=1}^{N}(Y_j - \bar{Y})^2 / N-1\right) + \left(\sum_{j=1}^{N}\left(\frac{U_{95}(Y_j)}{2}\right)^2 / N\right)}{N}}$$

Uncertainty Information from TUBITAK UME

w_sample= ((r_area - i_cal)/sl_cal) · m_is/m_sample
w_sample: mass fraction of aflatoxin in sample
r_area: area ratio native compound/internal standard
i_cal: intercept of calibration line
sl_cal: slope of calibration line
m_is: mass of internal standard added to sample
m_sample: mass sample

Uncertainty estimation was performed, using the following equation calculated according to EURACHEM CITAC Guide "Quantifying Uncertainty in Analytical Chemistry":

$$U = k\sqrt{u_{precision}^2 + u_{recovery}^2}$$

$$u_{precision} = \sqrt{S_r^2 + S_b^2}$$

$$S_r = \sqrt{MS_w}$$

$$S_b = \sqrt{(MS_b - MS_w)/n}$$

$$MS_b = SS_b/(p-1)$$

$$MS_w = SS_w/(N-p)$$

SS_b and SS_w are obtained from one way ANOVA

(p -1) and ($N - p$) are degrees of freedom obtained from one way ANOVA

Uncertainty Information from VNIIM

w- mass fraction of analyte in the sample, ng/g;
m_{is} - mass of internal standard added to sample before sample preparation, ng;
m - mass of the sample, g;
F - response factor.
$F=(S_{ancal}*m_{is})/(S_{iscal}*m_{an})$
m_{an}- mass of analyte in calibration solution;
m_{is} - mass of internal standard in calibration solution;
S_{ancal} - peak area for the analyte;
S_{iscal} - peak area for the internal standard

$$w_{an} = \frac{S_{an} \cdot m_{IS}}{S_{IS} \cdot F \cdot m}$$

Source of uncertainty	u, %			
	AF B1	**AF B2**	**AF G1**	**AF G2**
mass of sample (m)	0.0006	0.0006	0.0006	0.0006
preparation of calibration solution	1.29	3.14	1.85	3.27
concentration of reference standard solutions	0.87	0.87	0.87	0.87
RSD of F determination	2.65	2.69	1.45	9.85
mass of internal standard added to sample before extraction (mIS) (volume of IS solution added to sample)	0.58	0.9	1.45	0.96
RSD of results. %	2.15	5.9	4.66	23
comb.std uncertainty	**3.8**	**7.3**	**5.5**	**25**
expanded uncertainty (k=2)	**7.6**	**15**	**11**	**50**

APPENDIX H: Participants' Quantitative Results as Reported

The following are text excerpts and/or pictures of the quantitative results as provided by the participants in the reporting form. Information is grouped by participant and presented in alphabetized acronym order.

Quantitative Results from BAM

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	5.41	0.15	2.571	0.40
AFB2	0.66	0.03	2.571	0.08
AFG1	2.01	0.11	2.571	0.27
AFG2	0.22	0.01	2.571	0.03
Total AF	8.29	0.19	2.571	0.49

Quantitative Results from EXHM/GCSL-EIM

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	5.994	0.123	2.03	0.249
AFB2	0.871	0.022	2.11	0.047
AFG1	2.093	0.061	2.07	0.125
AFG2	0.264	0.01	2.20	0.022
Total AF	9.223	0.141	2.00	0.282

Quantitative Results from GLHK

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	5.8	0.5	2	1.1
AFB2	0.74	0.07	2	0.14
AFG1	2.0	0.2	2	0.5
AFG2	0.22	0.04	2	0.07
Total AF	8.7	0.6	2	1.2

Quantitative Results from INTI

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	5.17	0.33	2	0.66
AFB2	0.69	0.13	2	0.26
AFG1	2.5	0.07	2	0.14
AFG2	0.32	0.04	2	0.08
Total AF	8.68	0.57	2	1.14

Quantitative Results from KEBS

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	7.27	0.8	2	1.6
AFB2	0.60	0.1	2	0.2
AFG1	2.39	0.4	2	0.8
AFG2	0.06	0.01	2	0.02

Total AF	10.31	1.34	2	2.68

Quantitative Results from NIMT

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	6.6	0.40	2.03	0.9
AFB2	0.8	0.05	2.04	0.1
AFG1	2.6	0.18	2.10	0.4
AFG2	0.3	0.03	2.00	0.1
Total AF	10.3	0.44	2.57	1.2

Quantitative Results from NMISA

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	6.20	0.28	2	0.56
AFB2	0.755	0.040	2	0.080
AFG1	2.24	0.20	2	0.40
AFG2	0.214	0.025	2	0.049
Total AF	9.4	0.65	2	1.3

Quantitative Results from TUBITAK UME

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	5.72	0.33	2	0.66
AFB2	0.67	0.05	2	0.09
AFG1	2.16	0.15	2	0.30
AFG2	0.23	0.02	2	0.04
Total AF	8.78	0.35	2	0.70

Quantitative Results from VNIIM

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
AFB1	6.22	0.23	2	0.46
AFB2	0.81	0.06	2	0.12
AFG1	1.98	0.11	2	0.22
AFG2	0.15	0.04	2	0.08
Total AF	9.16	0.27	2	0.54