COMMISSION REGULATION (EU) No 519/2014

of 16 May 2014

amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules ([1]), in particular Article 11(4) thereof,

Whereas:

(1) Commission Regulation (EC) No 1881/2006 ([2]) provides for maximum limits for certain mycotoxins in certain foodstuffs.

(2) Sampling plays a crucial part in the precision of the determination of the levels of mycotoxins, which are heterogeneously distributed in a lot. It is therefore necessary to set out criteria which the sampling methods should fulfil.

(3) Commission Regulation (EC) No 401/2006 ([3]) establishes the criteria for the sampling for the control of the levels of mycotoxins.

(4) It is necessary to amend the rules concerning the sampling of spices in order to take into account the differences in particle size which leads to the heterogeneous distribution of mycotoxin contamination in spices. Furthermore it is appropriate to establish rules for the sampling of large lots in order to ensure a uniform enforcement approach across the Union. It is also appropriate to clarify which method of sampling has to be applied for the sampling of apple juice.

(5) The performance criteria for T-2 and HT-2 toxin need to be updated in order to take into account scientific and technological progress. Performance criteria for citrinin need to be established given the maximum level established for citrinin in food supplements based on rice fermented with the red yeast *Monascus purpureus*.

(6) For the analysis of mycotoxins, screening methodologies are used more and more. It is appropriate to establish criteria with which the screening methods have to comply with for use for regulatory purposes.

(7) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

Article 1

Regulation (EC) No 401/2006 is amended as follows:

(1) Annex I is amended as follows:

(a) In part B, footnote (1) is replaced by the following:

'(1) The sampling of such lots shall be performed in accordance with the rules set out in part L. Guidance for sampling large lots shall be provided in a guidance document available on the following website: http://ec.europa.eu/food/food/chemicalsafety/contaminants/guidance-sampling-final.pdf

([1]) OJ L 165, 30.4.2004, p. 1.
([2]) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (OJ L 364, 20.12.2006, p. 5).
([3]) Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs (OJ L 70, 9.3.2006, p. 12).

The application of sampling rules in accordance with EN ISO 24333:2009 or GAFTA Sampling Rules 124, applied by food business operators to ensure compliance with provisions in legislation is equivalent to the sampling rules set out in part L.

For the sampling of lots for *Fusarium*-toxins, the application of sampling rules in accordance with EN ISO 24333:2009 or GAFTA Sampling Rules 124, applied by food business operators to ensure compliance with provisions in legislation is equivalent to the sampling rules set out in part B;'

(b) In part B.2, Table 1 is replaced by the following table:

'Table 1

Subdivision of lots into sublots depending on product and lot weight

Commodity	Lot weight (tonne)	Weight or number of sublots	No incremental samples	Aggregate sample Weight (kg)
Cereals and cereal products	> 300 and < 1 500	3 sublots	100	10
	≥ 50 and ≤ 300	100 tonnes	100	10
	< 50	—	3-100 (*)	1-10

(*) Depending on the lot weight — see Table 2.'

(c) In part B.3, the following sentence is added at the end of the first indent:

'For lots > 500 tonnes, the number of incremental samples is provided for in part L.2 of Annex I.'

(d) In part D.2 the following sentence is added after the first sentence:

'This method of sampling is of also of application for the official control of the maximum levels established for ochratoxin A, aflatoxin B1 and total aflatoxins in spices with a relatively large particle size (particle size comparable with peanuts or larger e.g. nutmeg).';

(e) In part E, the first sentence is replaced by the following:

'This method of sampling is of application for the official control of the maximum levels established for ochratoxin A, aflatoxin B1 and total aflatoxins in spices except in cases of spices with a relatively large particle size (heterogeneous distribution of mycotoxin contamination).';

(f) In part I, the heading and the first sentence are replaced by the following:

'I. METHOD OF SAMPLING FOR SOLID APPLE PRODUCTS

This method of sampling is of application for the official control of the maximum levels established for patulin in solid apple products, including solid apple products for infants and young children.'

(g) In part I.1, second paragraph, the following sentences are deleted:

'In case of liquid products the lot shall be thoroughly mixed insofar as possible by either manual or mechanical means immediately prior to sampling. In this case, a homogenous distribution of patulin can be assumed within a given lot. It is therefore sufficient to take three incremental samples from a lot to form the aggregate sample.'

(h) New Parts L and M as set out in Annex I to this Regulation, are added.

(2) In Annex II, points 4.2 'General requirements', 4.3 'Specific requirements' and 4.4 'Estimation of measurement uncertainty, recovery calculation and reporting of results' are replaced by the text set out in Annex II to this Regulation.

Article 2

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

It shall apply from 1 July 2014.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 16 May 2014.

For the Commission

The President

José Manuel BARROSO

ANNEX I

'L. METHOD OF SAMPLING FOR VERY LARGE LOTS OR LOTS STORED OR TRANSPORTED IN A WAY WHEREBY SAMPLING THROUGHOUT THE LOT IS NOT FEASIBLE

L.1. **General principles**

In case the way of transport or storage of a lot does not enable to take incremental samples throughout the whole lot, sampling of such lots should preferably be done when the lot is in flow (dynamic sampling).

In the case of large warehouses destined to store food, operators should be encouraged to install equipment in the warehouse enabling (automatic) sampling across the whole stored lot.

When the sampling procedures as provided for in this part L are applied, the food business operator or his representative should be informed of the sampling procedure. If the sampling procedure is questioned by the food business operator or his representative, the food business operator or his representative shall enable the competent authority to sample throughout the whole lot at his/her own cost.

Sampling of a part of the lot is allowed, on the condition that the quantity of the sampled part is at least 10 % of the lot to be sampled. If a part of a lot of food of the same class or description has been sampled and identified as not satisfying Union requirements, it shall be presumed that the entire lot is also affected, unless further detailed assessment shows no evidence that the rest of the lot is unsatisfactory.

The relevant provisions, such as weight of the incremental sample, provided for in the other parts of this Annex are applicable for the sampling for very large lots or lots stored or transported in a way whereby sampling throughout the lot is not feasible.

L.2. **Number of incremental samples to be taken in the case of very large lots**

In the case of large sampled portions (sampled portions > 500 tonnes), the number of incremental samples to be taken = 100 incremental samples + √tonnes. However in case the lot is less than 1 500 tonnes and can be subdivided into sublots in accordance with the table 1 of part B and on the condition that the sublots can be separated physically, the number of incremental samples as provided for in part B have to be taken.

L.3. **Large lots transported by ship**

L.3.1. *Dynamic sampling of large lots transported by ship*

The sampling of large lots in ships is preferably carried out while the product is in flow (dynamic sampling).

The sampling is to be done per hold (entity that can physically be separated). Holds are however emptied partly one after the other so that the initial physical separation no longer exists after transfer into storage facilities. Sampling can therefore be performed based on initial physical separation or based on the separation after transfer into the storage facilities.

The unloading of a ship can last for several days. Normally, sampling has to be performed at regular intervals during the whole duration of unloading. It is however not always feasible or appropriate for an official inspector to be present for sampling during the whole operation of unloading. Therefore sampling of part of the lot is allowed to be undertaken (sampled portion). The number of incremental samples is determined by taking into account the size of the sampled portion.

Even if the official sample is taken automatically, the presence of an inspector is necessary. However if the automatic sampling is done with pre-set parameters which cannot be changed during the sampling and the incremental samples are collected in a sealed receptacle, preventing any possible fraud, then the presence of an inspector is only required at the beginning of the sampling, every time the receptacle of the samples needs to be changed and at the end of the sampling.

L.3.2. *Sampling of lots transported by ship by static sampling*

In cases where the sampling is done in a static way the same procedure as foreseen for storage facilities (silos) accessible from above has to be applied (see point L.5.1).

The sampling has to be performed on the accessible part (from above) of the lot/hold. The number of incremental samples is determined by taking into account the size of the sampled portion.

L.4. **Sampling of large lots stored in warehouses**

The sampling has to be performed on the accessible part of the lot. The number of incremental samples is determined by taking into account the size of the sampled portion.

L.5. **Sampling of storage facilities (silos)**

L.5.1. *Sampling of silos (easily) accessible from above*

The sampling has to be performed on the accessible part of the lot. The number of incremental samples is determined by taking into account the size of the sampled portion.

L.5.2. *Sampling of silos not accessible from above (closed silos)*

L.5.2.1. Silos not accessible from above (closed silos) with individual sizes > 100 tonnes

Food stored in such silos cannot be sampled in a static way. Therefore when the food in the silo has to be sampled and there is no possibility to move the consignment, the agreement has to be made with the operator that he or she has to inform the inspector about when the silo will be unloaded, partially or completely, in order to enable sampling when the food is in flow.

L.5.2.2. Silos not accessible from above (closed silos) with individual sizes < 100 tonnes

Contrary to the provision in part point L.1 (sampled part at least 10 %), the sampling procedure involves the release into a receptacle of a quantity of 50 to 100 kg and taking the sample from it. The size of the aggregate sample corresponds to the whole lot and the number of incremental samples relate to the quantity of the food from the silo released into the receptacle for sampling.

L.6. **Sampling of loose food in large closed containers**

Such lots can often only be sampled when unloaded. In certain cases it is not possible to unload at the point of import or control and therefore the sampling should take place when such containers are unloaded. The operator has to inform the inspector about the place and time of unloading the containers.

M. METHOD OF SAMPLING OF FOOD SUPPLEMENTS BASED ON RICE FERMENTED WITH RED YEAST *MONASCUS PURPUREUS*

This method of sampling is applicable to the official control of the maximum level established for citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus*.

Sampling procedure and sample size

The sampling procedure is on the supposition that the food supplements based on rice fermented with red yeast *Monascus purpureus* are marketed in retail packages containing usually 30 to 120 capsules per retail package.

Lot size (number of retail packages)	Number of retail packages to be taken for sample	Sample size
1-50	1	All capsules
51-250	2	All capsules
251-1 000	4	From each retail package taken for sample, half of the capsules
> 1 000	4 + 1 retail package per 1 000 retail packages with a maximum of 25 retail packages	≤ 10 retail packages: from each retail package, half of the capsules > 10 retail packages: from each retail package, an equal number of capsules is taken to result in a sample with the equivalent of the content of retail 5 packages'

ANNEX II

'4.2. **General requirements**

Confirmatory methods of analysis used for food control purposes shall comply with the provisions of items 1 and 2 of Annex III to Regulation (EC) No 882/2004.

4.3. **Specific requirements**

4.3.1. *Specific requirements for confirmatory methods*

4.3.1.1. Performance criteria

It is recommended that fully validated confirmatory methods (i.e. methods validated by collaborative trials for relevant matrices) are used where appropriate and available. Other suitable validated confirmatory methods (e.g. methods validated in-house on relevant matrices belonging to the commodity group of interest) may also be used provided they fulfil the performance criteria set out in the following tables.

Where possible, the validation of in-house validated methods shall include a certified reference material.

(a) Performance criteria for aflatoxins

Criterion	Concentration Range	Recommended Value	Maximum permitted Value
Blanks	All	Negligible	—
Recovery — Aflatoxin M1	0,01-0,05 mg/kg	60 to 120 %	
	> 0,05 mg/kg	70 to 110 %	
Recovery-Aflatoxins B_1, B_2, G_1, G_2	< 1,0 mg/kg	50 to 120 %	
	1-10 mg/kg	70 to 110 %	
	> 10 mg/kg	80 to 110 %	
Reproducibility RSD_R	All	As derived from Horwitz Equation (*)(**)	2 × value derived from Horwitz Equation (*)(**)
Repeatability RSD_r may be calculated as 0,66 times Reproducibility RSD_R at the concentration of interest.			

Note:

— Values to apply to both B_1 and sum of $B_1 + B_2 + G_1 + G_2$

— If sum of individual aflatoxins $B_1 + B_2 + G_1 + G_2$ are to be reported, then response of each to the analytical system must be either known or equivalent.

(b) Performance criteria for ochratoxin A

Level µg/kg	Ochratoxin A		
	RSD_r %	RSD_R %	Recovery %
< 1	≤ 40	≤ 60	50 to 120
≥ 1	≤ 20	≤ 30	70 to 110

(c) Performance criteria for patulin

Level µg/kg	Patulin		
	RSD_r %	RSD_R %	Recovery %
< 20	≤ 30	≤ 40	50 to 120
20-50	≤ 20	≤ 30	70 to 105
> 50	≤ 15	≤ 25	75 to 105

(d) Performance criteria for deoxynivalenol

Level µg/kg	Deoxynivalenol		
	RSD_r %	RSD_R %	Recovery %
> 100-≤ 500	≤ 20	≤ 40	60 to 110
> 500	≤ 20	≤ 40	70 to 120

(e) Performance criteria for zearalenone

Level µg/kg	Zearalenone		
	RSD_r %	RSD_R %	Recovery %
≤ 50	≤ 40	≤ 50	60 to 120
> 50	≤ 25	≤ 40	70 to 120

(f) Performance criteria for Fumonisin B_1 and B_2 individually

Level µg/kg	Fumonisin B_1 and B_2 individually		
	RSD_r %	RSD_R %	Recovery %
≤ 500	≤ 30	≤ 60	60 to 120
> 500	≤ 20	≤ 30	70 to 110

(g) Performance criteria for T-2 and HT-2 toxin individually

Level µg/kg	T-2 and HT-2 toxin individually		
	RSD_r %	RSD_R %	Recovery %
15-250	≤ 30	≤ 50	60 to 130
> 250	≤ 25	≤ 40	60 to 130

(h) Performance criteria for citrinin

Level µg/kg	Citrinin			
	RSD_r %	Recommended RSD_R %	Maximum allowed RSD_R %	Recovery %
All	0,66 × RSD_R	As derived from Horwitz Equation (*)(**)	2 × value derived from Horwitz Equation (*)(**)	70 to 120

(i) Notes to the performance criteria for the mycotoxins:

— The detection limits of the methods used are not stated as the precision values given at the concentrations of interest.

— The precision values are calculated from the Horwitz equation, in particular the original Horwitz equation (for concentrations $1{,}2 \times 10^{-7} \leq C \leq 0{,}138$) (*) and the modified Horwitz equation (for concentrations $C < 1{,}2 \times 10^{-7}$) (**).

(*) Horwitz equation for concentrations $1{,}2 \times 10^{-7} \leq C \leq 0{,}138$:

$RSD_R = 2^{(1-0.5\log C)}$

(ref: *W. Horwitz, L.R. Kamps, K.W. Boyer, J.Assoc.Off.Analy.Chem.,1980, 63, 1344)*

(**) Modified Horwitz equation (*) for concentrations $C < 1{,}2 \times 10^{-7}$:

$RSD_R = 22\ \%$

(ref: *M. Thompson, Analyst, 2000, 125, p. 385-386)*

Where:

— RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions [(sR/) × 100]

— C is the concentration ratio (i.e. 1 = 100g/100g, 0,001 = 1 000 mg/kg)

This is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

4.3.1.2. "Fitness-for-purpose" approach

For in-house validated methods, as an alternative, a "fitness-for-purpose" approach (***) may be used to assess their suitability for official control. Methods suitable for official control must produce results with a standard measurement uncertainty (u) less than the maximum standard measurement uncertainty calculated using the formula below:

$$Uf = \sqrt{(\mathrm{LOD}/2)^2 + (\alpha \times C)^2}$$

where:

— *Uf* is the maximum standard measurement uncertainty (µg/kg)

— LOD is the limit of detection of the method (µg/kg)

— α is a constant, numeric factor to be used depending on the value of C. The values to be used are set out in Table hereafter.

— C is the concentration of interest (µg/kg)

If the analytical method provides results with uncertainty measurements less than the maximum standard uncertainty the method shall be considered being equally suitable to one which meets the performance criteria given in point 4.3.1.1.

Table

Numeric values to be used for α as constant in formula set out in this point, depending on the concentration of interest

C (µg/kg)	α
≤ 50	0,2
51-500	0,18
501-1 000	0,15
1 001-10 000	0,12
> 10 000	0,1

(***) Ref: *M. Thompson and R. Wood, Accred. Qual. Assur., 2006, 10, p. 471-478.*

4.3.2. *Specific requirements for semi-quantitative screening methods*

4.3.2.1. Scope

The scope applies to bioanalytical methods based on immuno-recognition or receptor binding (such as ELISA, dip-sticks, lateral flow devices, immuno-sensors) and physicochemical methods based on chromatography or direct detection by mass spectrometry (e.g. ambient MS). Other methods (e.g. thin layer chromatography) are not excluded provided the signals generated relate directly to the mycotoxins of interest and allow that the principle described hereunder is applicable.

The specific requirements apply to methods of which the result of the measurement is a numerical value, for example a (relative) response from a dip-stick reader, a signal from LC-MS, etc., and that normal statistics apply.

The requirements do not apply to methods that do not give numerical values (e.g. only a line that is present or absent), which require different validation approaches. Specific requirements for these methods are provided in point 4.3.3.

This document describes procedures for the validation of screening methods by means of an inter-laboratory validation, the verification of the performance of a method validated by means of an inter-laboratory exercise and the single-laboratory validation of a screening method.

4.3.2.2. Terminology

Screening target concentration (STC): the concentration of interest for detection of the mycotoxin in a sample. When the aim is to test compliance with regulatory limits, the STC is equal to the applicable maximum level. For other purposes or in case no maximum level has been established, the STC is predefined by the laboratory.

Screening method: means method used for selection of those samples with levels of mycotoxins that exceed the screening target concentration (STC), with a given certainty. For the purpose of mycotoxin screening, a certainty of 95 % is considered fit-for-purpose. The result of the screening analysis is either "negative" or "suspect". Screening methods shall allow a cost-effective high sample-throughput, thus increasing the chance to discover new incidents with high exposure and health risks to consumers. These methods shall be based on bio-analytical, LC-MS or HPLC methods. Results from samples exceeding the cut-off value shall be verified by a full re-analysis from the original sample by a confirmatory method.

"Negative sample" means the mycotoxin content in the sample is < STC with a certainty of 95 % (i.e. there is a 5 % chance that samples will be incorrectly reported as negative).

"False negative sample" means the mycotoxin content in the sample is > STC but it has been identified as negative.

"Suspect sample" (screen positive) means the sample exceeds the cut-off level (see below) and may contain the mycotoxin at a level higher than the STC. Any suspect result triggers a confirmatory analysis for unambiguous identification and quantification of the mycotoxin.

"False suspect sample" is a negative sample that has been identified as suspect.

"Confirmatory methods" means methods that provide full or complementary information enabling the mycotoxin to be identified and quantified unequivocally at the level of interest.

Cut-off level: the response, signal, or concentration, obtained with the screening method, above which the sample is classified as "suspect". The cut-off is determined during the validation and takes the variability of the measurement into account.

Negative control (blank matrix) sample: a sample known to be free ([1]) of the mycotoxin to be screened for, e.g. by previous determination using a confirmatory method of sufficient sensitivity. If no blank samples can be obtained, then material with the lowest obtainable level might be used as long as the level allows the conclusion that the screening method is fit for purpose.

Positive control sample: sample containing the mycotoxin at the screening target concentration, e.g. a certified reference material, a material of known content (e.g. test material of proficiency tests) or otherwise sufficiently characterised by a confirmatory method. In the absence of any of the above, a blend of samples with different levels of contamination or a spiked sample prepared within laboratory and sufficiently characterised can be used, provided it can be proven that the contamination level has been verified.

4.3.2.3. Validation procedure

The aim of the validation is to demonstrate the fitness of purpose of the screening method. This is done by determination of the cut-off value and determination of the false negative and false suspect rate. In these two parameters performance characteristics such as sensitivity, selectivity, and precision are embedded.

Screening methods can be validated by inter-laboratory or by single laboratory validation. If inter-laboratory validation data is already available for a certain mycotoxin/matrix/STC combination, a verification of method performance is sufficient in a laboratory implementing the method.

4.3.2.3.1. Initial validation by single laboratory validation

Mycotoxins:

The validation shall be performed for every individual mycotoxin in the scope. In case of bio-analytical methods that give a combined response for a certain mycotoxin group (e.g. aflatoxins B_1, B_2, G_1 & G_2; fumonisins B_1 & B_2), applicability must be demonstrated and limitations of the test mentioned in the scope of the method. Undesired cross-reactivity (e.g. DON-3-glycoside, 3- or 15-acetyl-DON for immuno-based methods for DON) is not considered to increase the false negative rate of the target mycotoxins, but may increase the false suspect rate. This unwanted increasing will be diminished by confirmatory analysis for unambiguous identification and quantification of the mycotoxins.

Matrices:

An initial validation should be performed for each commodity, or, when the method is known to be applicable to multiple commodities, for each commodity group. In the latter case, one representative and relevant commodity is selected from that group (see table A).

Sample set:

The minimum number of different samples required for validation is 20 homogeneous negative control samples and 20 homogeneous positive control samples that contain the mycotoxin at the STC, analysed under intermediate precision (RSD_{Ri}) conditions spread over 5 different days. Optionally, additional sets of 20 samples containing the mycotoxin at other levels can be added to the validation set to gain insight to what extent the method can distinguish between different mycotoxin concentrations.

Concentration:

For each STC to be used in routine application, a validation has to be performed.

4.3.2.3.2. Initial validation through collaborative trials

Validation through collaborative trials shall be done in accordance with an internationally recognised protocol on collaborative trials (e.g. ISO 5725:1994 or the IUPAC International Harmonised Protocol) which requires inclusion of valid data from at least eight different laboratories. Other than that, the only difference compared to single laboratory validations is that the ≥ 20 samples per commodity/level can be evenly divided over the participating laboratories, with a minimum of two samples per laboratory.

([1]) Samples are considered free of analyte if the amount present in the sample does not exceed more than 1/5th of the STC. If the level can be quantified with a confirmatory method, the level must be taken into consideration for the validation assessment.

4.3.2.4. Determination of cut-off level and rate of false suspected results of blank samples

The (relative) responses for the negative control and positive control samples are taken as basis for the calculation of the required parameters.

Screening methods with a response proportional with the mycotoxin concentration

For screening methods with a response proportional with the mycotoxin concentration the following applies:

$$Cut\text{-}off = R_{STC} - t\text{-}value_{0,05} * SD_{STC}$$

R_{STC} = mean response of the positive control samples (at STC)

t-value: one tailed t-value for a rate of false negative results of 5 % (see table B)

SD_{STC} = standard deviationScreening methods with a response inversely proportional with the mycotoxin concentration

Similarly, for screening methods with a response inversely proportional with the mycotoxin concentration, the cut-off is determined as:

$$Cut\text{-}off = R_{STC} + t\text{-}value_{0,05} * SD_{STC}$$

By using this specific t-value for establishing the cut-off value, the rate of false negative results is by default set at 5 %.

Fitness for purpose assessment

Results from the negative control samples are used to estimate the corresponding rate of false suspect results. The t-value is calculated corresponding to the event that a result of a negative control sample is above the cut off value, thus erroneously classified as suspect.

t-value = (cut off − $mean_{blank}$)/$SD_{blank}$for screening methods with a response proportional with the mycotoxin concentration

or

t-value = ($mean_{blank}$ − cut off)/$SD_{blank}$for screening methods with a response inversely proportional with the mycotoxin concentration

From the obtained t-value, based on the degrees of freedom calculated from the number of experiments, the probability of false suspect samples for a one tailed distribution can either be calculated (e.g.. spread sheet function "TDIST") or taken from a table for t-distribution.

The corresponding value of the one tailed t-distribution specifies the rate of false suspect results.

This concept is described in detail with an example in Analytical and Bioanalytical Chemistry DOI 10.1007/s00216 -013-6922-1.

4.3.2.5. Extension of the scope of the method

4.3.2.5.1. Extension of scope to other mycotoxins:

When new mycotoxins are added to the scope of an existing screening method, a full validation is required to demonstrate the suitability of the method.

4.3.2.5.2. Extension to other commodities:

If the screening method is known or expected to be applicable to other commodities, the validity to these other commodities shall be verified. As long as the new commodity belongs to a commodity group (see Table A) for which an initial validation has already been performed, a limited additional validation is sufficient. For this, a minimum of 10 homogeneous negative control and 10 homogeneous positive control (at STC) samples shall be analysed under intermediate precision conditions. The positive control samples shall all be above the cut-off value. In case this criterion is not met, a full validation is required.

4.3.2.6. Verification of methods already validated through collaborative trials

For screening methods that have already been successfully validated through a collaborative laboratory trial, the method performance shall be verified. For this a minimum of 6 negative control and 6 positive control (at STC) samples shall be analysed. The positive control samples shall all be above the cut-off value. In case this criterion is not met, the laboratory has to perform a root-cause analysis to identify why it cannot meet the specification as obtained in the collaborative trial. Only after taking corrective action it shall re-verify the method performance in its laboratory. In case the laboratory is not capable to verify the results from the collaborative trial, it will need to establish its own cut-off in a complete single laboratory validation.

4.3.2.7. Continuous method verification/on-going method validation

After initial validation, additional validation data are acquired by including at least two positive control samples in each batch of samples screened. One positive control sample is a known sample (e.g. one used during initial validation), the other is a different commodity from the same commodity group (in case only one commodity is analysed, a different sample of that commodity is used instead). Inclusion of a negative control sample is optional. The results obtained for the two positive control samples are added to the existing validation set.

At least once a year the cut-off value is re-established and the validity of the method is re-assessed. The continuous method verification serves several purposes:

— quality control for the batch of samples screened

— providing information on robustness of the method at conditions in the laboratory that applies the method

— justification of applicability of the method to different commodities

— allowing to adjust cut-off values in case of gradual drifts over time.

4.3.2.8. Validation report

The validation report shall contain:

— A statement on the STC

— A statement on the obtained cut-off.

Note: The cut-off must have the same number of significant figures as the STC. Numerical values used to calculate the cut-off need at least one more significant figure than the STC.

— A statement on calculated false suspected rate

— A statement on how the false suspected rate was generated.

Note: The statement on the calculated false suspected rate indicates if the method is fit-for-purpose as it indicates the number of blank (or low level contamination) samples that will be subject to verification.

Table A

Commodity groups for the validation of screening methods

Commodity groups	Commodity categories	Typical representative commodities included in the category
High water content	Fruit Juices	Apple juice, grape juice
	Alcoholic beverages	Wine, beer, cider
	Root and tuber vegetables	Fresh ginger
	Cereal or fruit based purees	Purees intended for infants and small children

Commodity groups	Commodity categories	Typical representative commodities included in the category
High oil content	Tree nuts	Walnut, hazelnut, chestnut
	Oil seeds and products thereof	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame etc.
	Oily fruits and products thereof	Oils and pastes (e.g. peanut butter, tahina)
High starch and/or protein content and low water and fat content	Cereal grain and products thereof	Wheat, rye, barley, maize, rice, oats Wholemeal bread, white bread, crackers, breakfast cereals, pasta
	Dietary products	Dried powders for the preparation of food for infants and small children
High acid content and high water content (*)	Citrus products	
"Difficult or unique commodities" (**)		Cocoa beans and products thereof, copra and products thereof, coffee, tea Spices, liquorice
High sugar low water content	Dried fruits	Figs, raisins, currants, sultanas
Milk and milk products	Milk	Cow, goat and buffalo milk
	Cheese	Cow, goat cheese
	Dairy products (e.g. milk powder)	Yogurt, cream

(*) If a buffer is used to stabilise the pH changes in the extraction step, then this commodity group can be merged into one commodity group "High water content".
(**) "Difficult or unique commodities" should only be fully validated if they are frequently analysed. If they are only analysed occasionally, validation may be reduced to just checking the reporting levels using spiked blank extracts.

Table B

One tailed t-value for a false negative rate of 5 %

Degrees of Freedom	Number of replicates	t-value (5 %)
10	11	1,812
11	12	1,796
12	13	1,782
13	14	1,771
14	15	1,761
15	16	1,753
16	17	1,746
17	18	1,74
18	19	1,734

Degrees of Freedom	Number of replicates	t-value (5 %)
19	20	1,729
20	21	1,725
21	22	1,721
22	23	1,717
23	24	1,714
24	25	1,711
25	26	1,708
26	27	1,706
27	28	1,703
28	29	1,701
29	30	1,699
30	31	1,697
40	41	1,684
60	61	1,671
120	121	1,658
∞	∞	1,645

4.3.3. *Requirements for qualitative screening methods (methods that do not give numerical values)*

The development of validation guidelines for binary test methods is currently subject of various standardization bodies (e.g. AOAC, ISO). Very recently AOAC has drafted a guideline on this matter. This document can be regarded as the current state of the art in its field. Therefore methods that give binary results (e.g. visual inspection of dip-stick tests) should be validated according to this guideline

http://www.aoac.org/imis15_prod/AOAC_Docs/ISPAM/Qual_Chem_Guideline_Final_Approved_031412.pdf

4.4. **Estimation of measurement uncertainty, recovery calculation and reporting of results** ([1])

4.4.1. *Confirmatory methods*

The analytical result must be reported as follows:

(a) Corrected for recovery, the level of recovery being indicated. The correction for recovery is not necessary in case the recovery rate is between 90-110 %.

(b) As x +/– U whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

For food of animal origin, the taking into account of the measurement uncertainty can also be done by establishing the decision limit (CCα) in accordance with Commission Decision 2002/657/EC ([2]) (point 3.1.2.5 of Annex I — the case of substances with established permitted limit).

However if the result of the analysis is significantly (> 50 %) lower than the maximum level or much higher than the maximum level (i.e. more than 5 times the maximum level), and on the condition that the appropriate quality procedures are applied and the analysis serves only the purpose of checking compliance with legal provisions, the analytical result might be reported without correction for recovery and the reporting of the recovery rate and measurement uncertainty might be omitted in these cases.

([1]) More details on procedures for the estimation of measurement uncertainty and on procedures for assessing recovery can be found in the report "Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU food and feed legislation" — http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf

([2]) Commission Decision 2002/657/EC of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (OJ L 221, 17.8.2002, p. 8).

The present interpretation rules of the analytical result in view of acceptance or rejection of the lot apply to the analytical result obtained on the sample for official control. In case of analysis for defence or referee purposes, the national rules apply.

4.4.2. *Screening methods*

The result of the screening shall be expressed as compliant or suspected to be non-compliant.

"Suspected to be non-compliant" means the sample exceeds the cut-off level and may contain the mycotoxin at a level higher than the STC. Any suspect result triggers a confirmatory analysis for unambiguous identification and quantification of the mycotoxin.

"Compliant" means that the mycotoxin content in the sample is < STC with a certainty of 95 % (i.e. there is a 5 % chance that samples will be incorrectly reported as negative). The analytical result is reported as "< level of STC" with the level of STC specified.'