



Technical Specifications

# TS4.2 - Registered laboratories

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# 1. Introduction

## 1.1. Scope of this document

This document specifies the requirements and describes the registration process to become a GMP+ registered laboratory.

 **Helpful tip:**

See F0.2 *Definition list* for the definitions of some terms used in this document.

## 1.2. Application

This document applies to the GMP+ registered laboratory that analyses samples of feed on the following critical contaminants:

- a. Aflatoxin B1;
- b. sum of dioxins and dioxin-like PCBs / dioxins / dioxin-like PCBs / non-dioxin-like PCBs;
- c. heavy metals (Arsenic, Lead, Cadmium and Mercury) and fluorine;
- d. pesticides

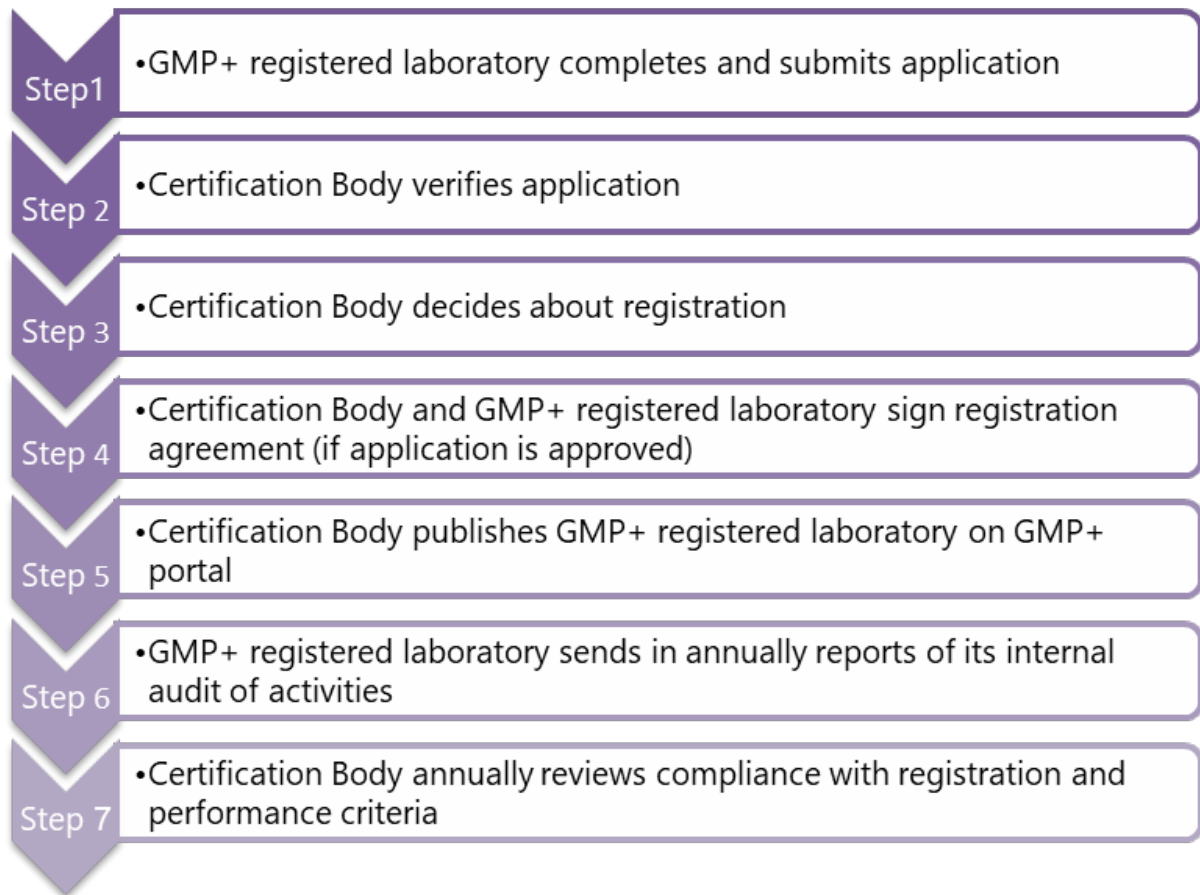
## 2. Requirements for GMP+ registration

A laboratory wishing to become a GMP+ registered laboratory must:

- a. Have an independently verified quality management system in place which:
  1. is accepted\* within the GMP+ Feed Certification scheme and;
  2. includes participation in proficiency testing in accordance with the requirements in Chapter 4 in this document and;
  3. includes continual improvement of its performance (for criteria see Chapter 5) in this document.
- b. Meet the performance criteria for GMP+ registered laboratories as laid down in Chapter 4 in this document.

\* See TS 1.2 *Purchase* for a list of accepted laboratory schemes.

### 3. Registration process



#### 3.1. Registration and Assessment

A laboratory wishing to become a GMP+ registered laboratory must submit an application to a Certification Body accepted by GMP+ International to perform audits for the scope 'Registered laboratory'.

The GMP+ registered laboratory must send the following documents to the Certification Body:

- a. A completed application form;
- b. A recent original excerpt of the registration at the official business registration authority.
- c. A copy of a valid certificate (if applicable) which is accepted within the GMP+ Feed Certification scheme with a copy of the most recent scope for certification and/or accreditation.
- d. A list with types of activities and associated matrices that fall under the scope of the relevant accepted laboratory scheme(s).
- e. The latest validation report of each analysis for which the GMP+ registered laboratory wants to become registered.
- f. The results of the latest proficiency testing programme for each analysis for which the GMP+ registered laboratory wants to become registered.

- g. In case of outsourcing of an analysis to a subcontracted GMP+ registered laboratory, documentation that demonstrates that the subcontracted GMP+ registered laboratory is registered for the analysis in question.
- h. In case of outsourcing, a contract between the GMP+ registered laboratory and the subcontracted GMP+ registered laboratory in which their cooperation is arranged.

Following submission of a fully-completed application and all the required documents, the Certification Body will carry out an assessment.

### 3.2. Decision about registration

The Certification Body will, within 6 weeks after receipt of the application, inform the GMP+ registered laboratory in writing about (non-) compliance with the requirements in this document.

If the application is approved, a registration agreement must be concluded, which must be signed by the Certification Body and the GMP+ registered laboratory.

After receiving a signed registration agreement by both parties, the Certification Body publishes all relevant information about the GMP+ registered laboratory in the GMP+ company database and issues a statement of compliance to the GMP+ registered laboratory.

### 3.3. Information Certification Body

The GMP+ registered laboratory must keep the Certification Body up to date about the information and evidence as requested in [Chapter 2](#) and [§ 3.1](#) above.

At least once a year, the GMP+ registered laboratory must send in the results of its internal audit of activities to the Certification Body to verify that its performance complies with the requirements as laid down in [Chapters 4](#) and [5](#) in this document.

## 4. Performance criteria for laboratory testing

Maximum limits of the performance criteria are noted in:	
mg/kg (ppm)*	Aflatoxin B1 Heavy metals Fluorin Pesticides
ng TEQ/kg*	Sum of dioxins and dioxin-like PCBs Dioxins Dioxin-like PCBs
µg/kg*	Non-dioxin-like PCBs

\* Derived to a moisture content of 12% (88% dry matter).

### Helpful tip:

See [Appendix 1](#) of this document to be used for calculating the performance criteria mentioned in this document.

### 4.1. Aflatoxin B1

Matrix	LOQ limit (mg/kg 88% DM)	Reproducibility	Bias	Expanded measurement uncertainty
<b>Feed materials</b>	0,001	25%	15%	60%
<b>Complementary and complete feed with the exception of:</b>	0,005	25%	15%	60%
Dairy cattle and calves	0,001	25%	15%	60%
Dairy sheep and lambs	0,001	25%	15%	60%
Dairy goats and kids	0,001	25%	15%	60%
Piglets	0,001	25%	15%	60%
Young poultry animals	0,001	25%	15%	60%
<b>Compound feed for:</b>				
Cattle (except dairy cattle and calves)	0,004	25%	15%	60%
Sheep (except dairy sheep and lambs)	0,004	25%	15%	60%
Goats (except dairy goats and kids)	0,004	25%	15%	60%

Pigs (except piglet)	0,004	25%	15%	60%
Poultry (except young animals)	0,004	25%	15%	60%

**+** Helpful tip:

Suitable methods for the analysis of Aflatoxin B1 generally use HPLC, combined with Fluorescence detection or MS detection. All quantitative analytical methods are allowed, provided the set limits are met. When an immuno-affinity column is used for clean up the recovery of Aflatoxin B1 should be higher than 80% and regularly checked for the matrices analysed. Since aflatoxin can be adsorbed by glass the use of acid-rinsed glassware is advised.

**+** Helpful tip:

Semi-quantitative methods like Thin Layer Chromatography (TLC), ELISA and others, can be used for screening purposes, confirmation of suspected non-compliance result is necessary. NEN-EN-ISO 6498 provides guidelines to the preparation of test samples. For the analysis of mycotoxins the laboratory sample is to be grinded in total. NEN-ISO 14718 provides a method for the determination of Aflatoxin B1 in feed by use of HPLC with fluorescence detection after post-column derivatization.

**+** Helpful tip:

Daylight should be excluded as much as possible during the whole procedure of transport of sample, sample preparation and analysis, since aflatoxin gradually breaks down under the influence of ultraviolet light. As the distribution of aflatoxin is extremely non-homogeneous, samples should be prepared - and especially homogenized - with extreme care e.g. by using the slurry method (ref).

## 4.2. Sum of dioxins and dioxin-like PCBs

Matrix	LOQ limit (ng TEQ/kg 88% DM)	Reproducibility	Bias	Expanded measurement uncertainty
<b>Feed materials</b>	0,2* limit from GMP+ TS1.5	15%	20%	50%
<b>Feed additives and premixtures</b>	0.3	15%	20%	50%
<b>Compound feed with the exception of:</b>	0.30	15%	20%	50%
<b>Compound feed for pet animals and fish</b>	0.75	15%	20%	50%

**+** Helpful tip:

The [EU Commission Regulation 2017/644](#) for the sampling and analysis of dioxins and dioxin-like PCB's describes in detail precautions and sets standards for laboratories analysing samples for dioxins. The regulation makes a difference between screenings methods and confirmatory methods. Screening methods are used for selection of those samples with levels of PCDD/Fs and dioxin-like PCBs that exceed the maximum levels or the action thresholds. They should allow a cost-effective high sample-throughput, thus increasing the chance to discover new incidents with high exposure and health risks to



consumers. Screening methods should be based on bioanalytical or GC-MS methods. Results from samples exceeding the cut-off value to check compliance with the maximum level should be verified by a full re-analysis from the original sample by a confirmatory method.

Confirmatory methods provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the maximum or in case of need at the action threshold. Such methods utilise gas chromatography/high resolution mass spectrometry (GC-HRMS) or gas chromatography/triple quadrupole mass spectrometry (GC-MS/MS).

 **Helpful tip:**

The mass spectrometric method to determine the tetra through octa dioxins should be based on United States Environmental Protection Agency protocols 1613 and the European harmonized protocol EN 16215:2012. These protocols describe the basis tuning and calibration of the hardware as well as criteria for identification and quantification with isotope dilutions and procedures for quality assurance and quality control. A standard QA programme should be included in the routine procedure e.g. determination of recovery of internal standards, accuracy of spiked samples and blanks.

 **Helpful tip:**

To express the toxic potency of the mixture of dioxins, the toxic equivalency factor (TEF) approach was used. A TEF value was assigned to the dioxins, which represents their relative toxic potency towards 2,3,7,8-TCDD, the most toxic dioxin congener which TEF value is 1.0. By multiplying the TEF value of each congener with the concentration of that congener in ng/kg product, the toxic value of that congener was calculated (ng TEQ/ kg product). Summarising the TEQ's of all congeners gives the total TEQ value in each sample.

 **Helpful tip:**

European legislation permits the use of bioanalytical methods such as the CALUX (Chemically Activated LUciferase gene eXpression) assay for screening of feed samples for elevated levels of PCDD/Fs and DL-PCBs. Screening results are compared with a cut-off concentration, enabling the analyst to decide over sample compliance and to identify those samples requiring further investigation by confirmatory analysis. In addition, screening results may give a numerical indication of the PCDD/F- and DL-PCB-TEQ-levels in the sample. Expression of bioanalytical results as BEQs is particularly helpful for the analyst performing the follow-up by a confirmatory method, but mandatory during the initial validation process.

 **Helpful tip:**

Laboratories applying bioassays within official control, or for other regulatory purposes, must be accredited according to EN ISO/IEC 17025. Methods must be validated thereby providing evidence for compliance with EU legal criteria as given in [Commission Regulations \(EU\) 2017/644](#) and [152/2009](#) (including amendments). The proficiency of the laboratory should be proven by internal and external quality control measures. Continuous and successful participation in interlaboratory studies based on analyses of PCDD/Fs and DL-PCBs in the relevant feed / food matrices is mandatory.

 **Helpful tip:**

A screening method in principle classifies a sample as compliant or suspected to be non-compliant. For this, the calculated BEQ level is compared to the cut-off value. Samples below the cut-off value are declared compliant, samples equal or above the cut-off value as suspected to be non-compliant, requiring analysis by a confirmatory method. The set limits are applicable for both kind of methods.

Confirmatory methods should be applied in case of results exceeding the standards set in GMP+ TS1.5 *Specific feed safety limits*.

individual limits for dioxins or PCB's are set the laboratory has to provide evidence for the different sets of performance criteria.

The laboratory will include lower bound TEQ-values as well as upper bound TEQ-values. A sample exceeding the legal limits is considered confirmed when the difference between the lower bound and upper bound TEQ-value is <20%.

The lot is non-compliant with the maximum level as laid down in Regulation (EC) No 1881/2006, if the upperbound analytical result is obtained with a confirmatory method and is confirmed by duplicate analysis.

A duplicate analysis is necessary to exclude the possibility of internal cross-contamination or an accidental mix-up of samples. The first analysis, taking into account the measurement uncertainty is used for verification of compliance.

In case the analysis is performed in the frame of a contamination incident, confirmation by duplicate analysis might be omitted in case the samples selected for analysis are through traceability linked to the contamination incident.

Performance criteria as given in tables above are all based on total TEQ upperbound.

## 4.3. Dioxins

Matrix	LOQ limit (ng TEQ/kg 88% DM)	Reproducibility	Bias	Expanded measurement uncertainty
Feed materials	0,2* limit from GMP+ TS1.5	15%	20%	50%
Feed additives and premixtures	0.2	15%	20%	50%
Compound feed with the exception of:	0.15	15%	20%	50%
Compound feed for pet animals and fish	0.25	15%	20%	50%

### Helpful tip:

The helpful tips 1-6 as mentioned in [§ 4.2](#) may also be helpful when performing analyses on dioxin.

### 4.3.1. LOQ for Bioassays

For a bioanalytical screening method, establishment of the LOQ is not an indispensable requirement but the method must prove that it can differentiate between the blank and the cut-off value. When providing a BEQ- level, a reporting level must be established to deal with samples showing a response below this level. The reporting level:

- must be demonstrated to be different from procedure blank samples at least by a factor of three, with a response below the working range;

b. must therefore be calculated from samples containing the target compounds around the required minimum level, and not from a S/N ratio or an assay blank.

LOQ for Bioassay, however, must be as such that a BEQ level corresponding to 2/3 of the maximum level must serve as the most suitable cut-off value ensuring a false-compliant rate below 5% and an acceptable rate for false-non-compliant results.

#### 4.4. Dioxin-like PCBs

Matrix	LOQ limit (ng TEQ/kg 88% DM)	Reproducibility	Bias	Expanded measurement uncertainty
Feed materials	0.4	15%	20%	50%
Feed additives and premixtures	0.4	15%	20%	50%
Compound feed with the exception of:	0.15	20%	20%	50%
Compound feed for pet animals and fish	0,5	10%	20%	45%

#### Helpful tip:

The helpful tips 1-6 as mentioned in [§ 4.2](#) may also be helpful when performing analyses on dioxin-like PCBs.

#### 4.5. Non-dioxin-like PCBs

Matrix	LOQ limit (µg/kg 88% DM)	Reproducibility	Bias	Expanded measurement uncertainty
Feed materials	3.33	15%	20%	50%
Feed additives and premixtures	3.33	15%	20%	50%
Compound feed with the exception of:	3.33	15%	20%	50%
Compound feed for pet animals and fish	10	15%	20%	50%

## 4.6. Heavy metals and Fluorine

Matrix	LOQ limit (mg/kg 88% DM)	Reproducibility	Bias	Expanded measurement uncertainty
<b>Feed materials a.o minerals:</b>				
Arsenic (As) total	0.4	25%	15%	60%
Lead (Pb)	1.0	20%	15%	50%
Cadmium (Cd)	0.2	20%	15%	50%
Mercury (Hg)	0.02	25%	20%	65%
Fluorine (F)	30	10%	15%	35%
<b>Feed additives and premixtures <sup>1</sup>:</b>				
Arsenic (As) total	6.0	20%	10%	45%
Lead (Pb)	6.0	15%	10%	40%
Cadmium (Cd)	0.4	15%	10%	40%
<b>Complementary and complete feed:</b>				
Arsenic (As) total	0.4	25%	10%	60%
Lead (Pb)	1.0	20%	10%	45%
Cadmium (Cd)	0.1	20%	10%	45%
Mercury (Hg)	0.02	25%	20%	60%
Fluorine (F)*	6.0	10%	15%	35%

1. [1] Because of the absence of feed safety limits, no performance criteria are established for analysing mercury and fluorine in feed additives and premixtures.

\* Although not being a heavy metal LOQs for Fluorine are also taken into account.

### + Helpful tip:

There are many suitable methods for the analysis of legally regulated heavy metals; Cadmium (Cd), Arsenic (As), Lead (Pb) and Mercury (HG) such as:• Inductively coupled plasma atomic emission spectroscopy (ICP-OES)• Inductively coupled plasma mass spectroscopy (ICP-MS)• Graphite furnace atomic absorption spectrometry (GF-AAS).For ICP-OES the limit of quantification is in general at the mg/kg, while GF-AAS or ICP-MS for most of the heavy metals the limits of quantification are much lower. These methods can be applied on in general small amounts of samples which needs to be well homogenized (<0,5mm<sup>2</sup>) followed by complete digestion of the matrix with e.g. HNO<sub>3</sub>.In case of ICP-MS it is strongly advised to use the so called collision/reaction cell technology to remove polyatomic Interferences such as ArCl<sup>+</sup> which otherwise might result in false positive results.

### + Helpful tip:

For the determination of Mercury specific methods can be used for example: based on sample thermal decomposition, mercury amalgamation and atomic absorption detection. The limit of quantification using this technique is very low. As the sample intake is very low mostly up to 0,1 gram the sample should also for these methods well homogenized (<0,5mm<sup>2</sup>). From the well homogenized sample a subsample in general 0,1 to 1 gram should be digested in acid or in a muffle furnace. All quantitative analytical methods are allowed, provided the set limits are met. The method detection limit for each method of determination and for each element is dependent on the sample matrix as well as of the instrument and technology used.

**+** Helpful tip:

Fluorine can be determined after treatment with hydrochloric acid using spectroscopy or an Ion Selective Electrode.

**+** Helpful tip:

In case of a result of analysis above or around the maximum limit it is advised to repeat the analysis using fresh samples and quantification based on "standard addition" by spiking two subsamples on two different levels e.g. one just 0,5\* ML ('maximum limit') and one 1,5 \*ML . By using standard addition the sample specific matrix effects are minimized.

## 4.7. Pesticides

The laboratory must demonstrate compliance with the performance criteria for the analysis of all pesticide parameters included in the table below in order to become registered.

Parameters	LOQ (mg/kg 88% DM)	Reproducibility	Recovery <sup>1</sup>	Expanded measurement uncertainty
Aldrin	0.01	20%	70-120%	50%
Dieldrin	0.01	20%	70-120%	50%
Chlordane (sum of cis- and trans-isomers and of oxychlordane, expressed as chlordane)	0.01	20%	70-120%	50%
DDT (sum of p,p'-DDT, o,p'-DDT, p-p'-DDE and p,p'-TDE (DDD) expressed as DDT)	0.05	20%	70-120%	50%
Endosulfan (sum of alpha- and beta-isomers and endosulfan-sulphate expressed as endosulfan)	0.01	20%	70-120%	50%
Endrin (sum of endrin and of delta-keto-endrin, expressed as endrin)	0.01	20%	70-120%	50%
Heptachlor (sum of heptachlor and of heptachlorepoxyde, expressed as heptachlor)	0.01	20%	70-120%	50%
Hexachlorobenzene (HCB)	0.01	20%	70-120%	50%

Parameters	LOQ (mg/kg 88% DM)	Reproducibility	Recovery <sup>1</sup>	Expanded measurement uncertainty
Hexachlorocyclohexane (HCH)			70-120%	50%
- alpha-isomers	0.01	20%	70-120%	50%
- beta-isomers	0.01	20%	70-120%	50%
- gamma-isomers	0.01	20%	70-120%	50%
Fipronil (sum fipronil + sulfone metabolite (MB46136) expressed as fipronil)	0.005	20%	70-120%	50%
Chlorpyrifos-methyl	0.01	20%	70-120%	50%
Pirimiphos-methyl	0.01	20%	70-120%	50%
Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))	0.05	20%	70-120%	50%
Tebuconazole	0.02	20%	70-120%	50%
Deltamethrin (cis-deltamethrin)	0.02	20%	70-120%	50%
Chlorpropham	0.01	20%	70-120%	50%
Chlorpyrifos-ethyl	0.01	20%	70-120%	50%
Diphenylamine	0.05	20%	70-120%	50%
Malathion (sum of malathion and malaoxon expressed as malathion)	0.02	20%	70-120%	50%
Permethrin (sum of isomers)	0.05	20%	70-120%	50%

<sup>1</sup> In exceptional cases, mean recovery rates outside the range of 70-120 % can be accepted if they are consistent (RSD ≤ 20 %) and the basis for this is well established (e.g. due to analyte distribution in a partitioning step), but the mean recovery must not be lower than 30 % or above 140 %.

 **Helpful tip:**

Support document S9.13 Managing pesticide residues in feed can be consulted in order to find the correct MRL. This document describes and answers the questions from GMP+ certified companies participants about the requirements in the GMP+ FC scheme regarding the applicable MRLs for pesticides in feed.

 **Helpful tip:**

The performance criteria set in this chapter are based on the criteria as laid down in [SANTE 12682/2019](#).

 **Helpful tip:**

The majority of the pesticides to be controlled can be analyzed using the so called QuEChERS (Quick Easy Cheap Effective Rugged Safe) method. This method is based on extraction with acetonitrile in the

presence of water followed by a separation, controlled by a mixture of MgSO<sub>4</sub> and NaCl, of the water-phase. Subsequently the acetonitrile phase can be analyzed using GC-MS and LC-MS/MS covering a wide range of pesticides with different physical and chemical properties. For some pesticides a specific method (single compound) is needed e.g. dithiocarbamates. For very polar compounds specific methods based on the so called QuPpe (Quick Polar Pesticides Method). See also : [http://www.crl-pesticides.eu/docs/public/tmpl\\_article.asp?CntID=887&LabID=200&Lang=EN](http://www.crl-pesticides.eu/docs/public/tmpl_article.asp?CntID=887&LabID=200&Lang=EN)

## 5. Improvement

### 5.1. General

The GMP+ registered laboratory must participate in proficiency tests to proof its daily competence. The proficiency testing must be used to monitor laboratories' continual performance.

The proficiency testing must comply at least with the requirements in § 5.2 – 5.5.

### 5.2. Proficiency testing

Once a year, the GMP+ registered laboratory must take part in a proficiency testing for each analysis for which it is registered. The GMP+ registered laboratory must ensure that the provider of the proficiency test conducts the proficiency test in accordance with\*\* ISO 17043.

In case no proficiency test is available, the performance of the analysis must be demonstrated on basis of:

- a. a certified reference material or;
- b. if this is not available:
  1. a reference material from past proficiency tests or;
  2. a spiked sample.

*\*\* This means the provider does not have to be accredited, but must conduct the proficiency test in line with ISO 17043.*

### 5.3. Planning

Once a year the GMP+ registered laboratory must make a plan in which proficiency tests it will participate. This plan must be retained as documented information.

### 5.4. Administration

The GMP+ registered laboratory must retain the results of a proficiency test as documented information for at least 3 years.

The GMP+ registered laboratory's result must be expressed into a 'z-score'.

#### Helpful tip:

The 'z-score' reflects:

- a. the actual accuracy achieved (the difference between the laboratory's result and the accepted true or consensus value) and;
- b. the judgement of the provider of the proficiency test of what degree of accuracy is fit for purpose.



## 5.5. Interpretation of proficiency testing results

The GMP+ registered laboratory must interpret the results of the proficiency test based on the limits in table below.

Z-score	Assessment
$Z \leq 2$	Satisfactory
$2 < Z < 3$	Questionable
$Z \geq 3$	Unsatisfactory

In case the z-score is assessed as questionable or unsatisfactory, the GMP+ registered laboratory must:

- a. investigate the probable cause(s) of deviation, and
- b. implement corrective action, and
- c. verify that the corrective action lead to a satisfactory performance.

## Appendix 1 Calculations

The following calculations regarding the performance criteria apply:

Performance criteria <sup>2</sup>	Calculation
Limit of detection (LOD)	$LOD = 3 \times s_R$
Limit of quantification (LOQ)	$LOQ = 6 \times s_R$
Reproducibility	$R = \sqrt{\frac{\sum_{i=1}^n (x_{i1} - x_{i2})^2}{2n}}$
Bias	$\delta = \bar{x} - c_{ref}$
Expanded measurement uncertainty	$U = 2 \times \sqrt{s_R^2 + \delta^2}$

2. [2] Definitions and calculations are derived from NEN 7777: Prestatiekenmerken van meetmethoden and NEN 7779: Meetonzekerheid

$s_R$	the standard deviation in blanc samples under reproducibility circumstances
$R$	Reproducibility (determined in this standard at MRL level)
$\delta$	bias
$U$	expanded measurement uncertainty
$n$	number of analysis
$x$	concentration of the component
$\bar{x}$	mean concentration of the component analysed in the reference material
$c_{ref}$	the assigned value of the component in reference material

The average bias of the method is obtained from certified reference material or from materials from proficiency tests with an assigned (consensus) value. Bias based on addition is acceptable when interference with the matrix is not expected. Addition to at least eight different sample matrices is used when calculating the bias from the calculated recovery (accuracy).



## Feed Support Products

That was a lot of information to digest and one might ask, what is the next step? Luckily we can offer support for the GMP+ Community when doing this. We provide support by means of various tools and guidances but as each company has a shared responsibility to feed safety, and therefor tailor-made solutions cannot be offered. However, we do help by explaining requirements and provide background information about the requirements.

We have developed various supporting materials for the GMP+ Community. These include various tools, ranging from Frequently Asked Questions (FAQ) lists to webinars and events.

### **Supporting materials related to this document (Guidelines and FAQ's)**

We have made documents available which give guidance to the GMP+ requirements as laid down in the module GMP+ FSA and GMP+ FRA. These documents give examples, answers to frequently asked questions or background information.

Where to find more about the GMP+ International Feed Support Products

Fact sheets

More information: [Fact Sheets](#)

Review fact sheets: [GMP+ Portal](#)

# We enable every company in the feed chain to take responsibility for safe and sustainable feed.

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