VLOG	Recommendations for GMO Analyses According to the VLOG Standard	

The following requirements are currently recommendations for participants in the VLOG system and will be integrated – in an amended or extended manner, if necessary – in the next version of the VLOG Standard, and will then be valid as mandatory requirements.

1. Requirements for commissioning an analysis

The client commissioning the GMO analysis undertakes to:

- to regularly examine the accreditation of the commissioned laboratory pursuant to DIN EN ISO/IEC 17025 (cf. 2.1) at least once a year until 31 December 2018; and
- to check the VLOG recognition of the commissioned laboratory (cf. 2.1) on 1 January 2019 and regularly thereafter, at least once per year

When commissioning a laboratory, the following information must be indicated in the order or other documents having similar effect, and submitted to the laboratory:

- Order of GMO analyses according to this catalogue of requirements
  - Composition of the sample:
    If containing soy, corn, canola and/or rice single feed or ingredients, it must be indicated in what form these are contained (e.g. soy as soy extraction meal). Copies of the feed delivery bills / shipping documents / declarations are to be sent to the laboratory along with the samples.

Upon receipt of the analysis results, the client is to verify that the laboratory confirms compliance with the requirements mentioned in Chapter 2. This may be done for every analysis result in the examination report or in a separate confirmation that is issued by the laboratory once a year.

2. Requirements for laboratories

For certification according to the VLOG standard, only analysis results obtained according to the following requirements may be recognised.

General requirements and recognition by VLOG

- The laboratories must be accredited according to DIN EN ISO/IEC 17025 (in its most recent version) for all qualitative and quantitative GMO examination parameters. This may be in the form of a flexible accreditation for the entire field or separately for all procedures to be carried out.
- The analysis of the samples must be carried out entirely in the commissioned laboratory; sub-contracting to other laboratories is not permitted.

Laboratories must be recognised by VLOG by 1 January 2019 at the latest. Laboratories may apply for VLOG recognition as of 1 April 2018. The laboratories must prove to VLOG in writing that they comply with the requirements mentioned in Chapters 2.1, 2.2, and 3[1].

After being recognised, the laboratory must prove to VLOG that it regularly and successfully participates in proficiency tests, with reliable results for GMO testing. The laboratory must prove to VLOG in the first quarter for the preceding year, without being asked,

[1] Deviations from the qualifications must be justified in detail in the application and require written consent by the VLOG office.

- a proficiency tests regarding GMOs for quantitative results with a good z-score (+/- 2); and
- a proficiency test regarding GMOs for qualitative results (100% correct positive or negative results)

for feed or plant-based raw materials or plant-based processed products, indicating the z-score,

Furthermore, in the event of re-accreditation or a change to the scope of the accreditation, the laboratory must submit to VLOG the updated accreditation certificate according to DIN EN ISO/IEC 17025 within 4 weeks.

VLOG reserves the right to check compliance of requirements with an audit of the laboratory.

Methodological requirements

DIN standards and protocols of the Joint Research Centre (JRC; http://gmocrl.jrc.ec.europa.eu/StatusOfDossiers.aspx) should be used (if available). For methods from other sources, the laboratory must verify that similar minimum requirements are fulfilled.

Analytical process

Milling:

Depending on the sample matrix, the following minimum amounts of sample material are to be completely milled in each case:

- Feed: min. 400 g, max. 1 kg, entirely milled
- Raw material (whole corn kernels, soy beans or canola grains, among other): at least 3000 grains or approx. the respectively corresponding sample amount (corn at least 1000 g; soy at least 700 g, canola at least 60 g), entirely milled

DNA extraction:

At least two DNA extractions from each sample will be carried out after every milling /homogenisation. The weight should be at least 2000 mg for feed, seeds and materials that are suspected of being not being homogenously distributed. In exceptional cases (for otherwise non-extractable material), the weight may be only 500 mg.

PCR analysis:

Real-time PCR methods with probe technology (45 cycles) are recommended. When using conventional endpoint PCR methods, an additional confirmation reaction must be carried out (e.g. real-time PCR with probe technology, restriction analysis or sequencing).

The requirements for the scope of the analysis in Chapter 3 must be met.

Analytical quality control

All quality controls according to the relevant ISO and DIN standards must show the results required by these norms. The laboratory must ensure that the measurement results are not affected by any inhibitory effects. If the measurements are so different from the control values that the tolerance limits set by the laboratory for deviations or quality specifications are exceeded, the PCR process must be repeated.

To prevent systematic errors, instability of reagents etc., methods for regularly carrying out and documenting QC measures must be established and implemented (e.g. control charts).

Approval of analytical results

The results must be approved according to the four-eye principle by an authorised person.

Requirements for examination reports

Aside from the information required by DIN EN ISO 24276, DIN EN ISO 21569 and DIN EN ISO 21570, examination reports must contain at least the following information:

- Quantity of sample submitted and milled
- Quantity of sample used in the DNA extraction
- Exact description of the sample
- Detection limits (LOD in % or as copy number of target)
- Method applied
- Result of the examination
- Inaccuracy of the procedure
- Confirmation that the result was determined according to the requirements of the VLOG Standard. In the alternative, this confirmation may take place in a separate letter to be submitted to the certification body once a year
- Additionally for identification/quantification:
    - Warning if the amount of species-specific DNA is not sufficient for quantitative statements regarding the relevant threshold value (0.1 % or 0.9 % GMO DNA)
    - When quantifying, to indicate the average deviation of the sub-samples (at least double preparation); indicating the pLOQ is recommended

Interpretation of the measurement results – criteria for monitoring and assessing

There must be a conclusive assessment for each sample on the examination report if the sample complies with the requirements of the VLOG Standard for the parameter analysed.

For the assessment, the standard deviation is stringently to be considered in order to account for the non-homogeneous distribution of GMOs in feed or food. In keeping with Regulation (EC) No. 691/2013, the analysed GMO quantity, after deduction of the expanded measuring error margin, is used for the assessment.

Chapter 5 and Annexes 1 and 2 of the "Guideline for the Control of GMOs in Feeds"[2] must be respected for the assessment of feed.

If a conclusive assessment of the measurement results is not possible, this must be appropriately represented in the examination report (note in the event of limited analysability of the sample, indication of the practical LOD, missing information for single-component feeds).

[2] Guideline for the Control of GMOs in Feed (German: Leitfaden zur Kontrolle von GVO in Futtermitteln – version of November 2011). Monitoring of the production, of treatment, of use and of bringing to the market of feed in connection with genetically modified organisms (GMOs). … Compiled by the project group 'GMOs in Feed' of the Feed Working Group within the Working Group for Consumer Protection of the German Länder (German: LAV – Länderarbeitsgemeinschaft Verbraucherschutz) with participation of the Federal Government and the Association of German Agricultural Analytic and Research Institutes (VDLUFA), http://www.ohnegentechnik.org/Leitfaden_Futtermittel

Requirements for the scope of analysis

It must be noted that, regarding the minimum requirements for the scope of analysis in Chapter 3.1 to 3.6, not all GMOs were taken into account that are authorised in the EU or tolerated for feed within the meaning of EU Regulation No. 619/2011. Furthermore, GMOs not authorised in the EU are not part of the minimum requirements. In the event of an examination of the marketability and proper labelling of a feed, other GMOs would be taken into account (this includes other GMOs authorised in the EU, GMOs tolerated in feeds pursuant to EU Regulation No. 619/2011, and GMOs not authorised in the EU).

In consultation with laboratories, VLOG regularly checks and updates the following minimum requirements in Chapter 3.1 to 3.6 concerning the scope of analysis of raw materials and feeds. In the event of developments that other GMOs become relevant (e.g. RASFF reports), VLOG will provide its members and VLOG-certified companies with corresponding analysis requirements/guidelines in a timely manner.

This does not mean, however, that the companies participating in the VLOG system are dispensed from their own due diligence obligations to regularly check and, if necessary, update the scope of analysis.

Minimum requirements for raw soy materials / soy-based single-component feed

Determination and assessment of the summation value of the most relevant soy GMOs:

- Quantification of GTS 40-3-2 (RRS- 1)
- Quantification of MON89788 (RRS-2)
- Qualitative detection of A2704-12
  In the event of positive result for A2704, the quantity of this GMO can, for example, be estimated using the ΔΔct method or similar method ensuring that sufficient quantities of species DNA are present. For values over 0.1%, a quantification must be carried out.

Alternately, the laboratory may work with screening parameters that detect at least the GMOs mentioned. In subsequent identification / quantification of positive findings, at least all GMOs (if corresponding elements are positive) mentioned here must be quantified.

Minimum requirements for raw corn materials / corn-based single-component feed

1. **Screening for 35S Promoter (p35S) and NOS Terminator (tNOS).**
   Other screening elements can be implemented to narrow the corresponding GMO down.
2. **If positive:** Analysis at least for NK603, TC1507, MON810, MON89034 + RRS-1

If using the positive screening parameters, one or more of these GM corn types can be ruled out, then the same number of commercialised GM corn types that come into question must be searched for instead.

Positive screening results must be clarified; if none of the 4 GM corn types are positive, other GM types must be analysed.

3. **Determining the summation value of the corn GMO**
   Identified varieties must be quantified if the estimation of the concentration, when using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

RRS-1 positive:
Estimating the soy mass and assessing the amount of soy: Is it a relevant amount or minimal traces? If a botanical contamination containing GMO is determined, an assessment according to the official guidleine[2] must take place.

Minimum requirements for raw canola materials / canola-based single component feeds

1. **Triple screening** that detects all relevant GM canola varieties (e.g. tNOS, pat gene (or LibertyLink construct), CTP2-CP4epsps (or pFMV))

2. **ID depending on positive screening results**

- tNOS positive: at least RRS + bar gene for MS8 / RF3 or both directly
- pat gene / LibertyLink positive: at least canola T45
- CTP2-CP4epsps / pFMV positive: at least GT73

3. **Determining the summation value of GM canola**
   Identified GM canola varieties must be quantified if the estimation of the quantity, when using, for example, the ΔΔct method or another method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

Positive screening results must be clarified.

If no canola GMO is detected, the presence of a botanical contaminant containing GMO with soya or corn GMO must be clarified (estimation and assessment of masses). Is it a relevant quantity or minimal traces? If a botanical contamination containing GMO is determined, an assessment according to the official guidline[2] must take place.

Minimum requirements for compound feed containing soya

Determination and assessment of the summation value of the most relevant GMOs:

Soy:

- Quantification of GTS 40-3-2 (RRS- 1)
- Quantification of MON89788 (RRS-2)
- Qualitative detection of A2704-12
  In case of positive result for of A2704, the quantity of this GMO can, for example, be estimated using the ΔΔct method or a similar method ensuring that sufficient quantities of species DNA are present. For values over 0.1%, a post-quantification must be carried out.

In case of limited analysability of the soya ingredient, the practical LOD must be indicated.

For corn ingredient:

Additional qualitative detection of the 3 commercialised corn varieties: NK603, TC1507, MON810

In case of positive result, the quantity of this GMO can, for example, be estimated using the ΔΔct method or a similar method ensuring that sufficient quantities of species DNA are present. For values over 0.1%, a post-quantification of the GMOs detected must be carried out.

In the event of limited analysability of the corn ingredient, the practical LOD must be indicated.

For canola ingredient:

Additional qualitative detection of GT73

In case of positive identification, quantification of GT73 must take place if the estimation of the quantity using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

In case of limited analysability of the canola ingredient, the practical LOD must be indicated.

Alternately, the laboratory may also work with screening parameters that detect at least the GMOs mentioned (soy, canola, corn). In subsequent identification / quantification of positive results, at least all GMOs (if corresponding elements are positive) mentioned here must be identified and, if necessary, quantified.

Minimum requirements for soy-free compound feed

Determination and assessment of the summation value of the most relevant GMOs:

Estimating the soy mass:

In a first step, the mass of soy in the feed is estimated. For quantities over 0.9%, the quantity of soy GM must be determined (cf. Minimum requirements for feed containing soy) and an assessment according to the official guideline[2] must take place.

For canola ingredient:

Qualitative evidence of canola GT73 + canola MS8 or canola RF3 (or bar gene)

In the event of positive identification, quantification of GMO or GMOs found must take place if the estimation of the quantity when using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

In the event of limited analysability of the corn ingredient, the practical LOD must be indicated.

For corn ingredient:

Qualitative evidence of 3 corn varieties used commercially: NK603, TC1507, MON810

In the event of positive identification, quantification of GMO or GMOS found must take place if the estimation of the quantity when using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

In the event of limited analysability of the corn ingredient, the practical LOD must be indicated.

Alternately, the laboratory may work with screening parameters that detect at least the GMOs mentioned (soy, canola, corn). In subsequent identification / quantification of positive results, at least all GMOs (if corresponding elements are positive) mentioned here must be identified and, if necessary, quantified.

Minimum requirements for rice and rice products

VLOG has already published a document that contains the requirements for the scope of the GMO analysis of rice and rice products[3].

Other products / raw materials

The strategies for analysing GMOs in other single-component feeds, raw materials, (food) ingredients, intermediate products or foods must continue to be agreed upon with the commissioned laboratory, taking into account the composition and origin of the products.

[3] "Requirements for 'Ohne Gentechnik' Certification of Rice and Rice Products" (as of 16 September 2016) from the Verband Lebensmittel ohne Gentechnik e.V. (VLOG), http://www.ohnegentechnik.org/fileadmin/ohne-gentechnik/fuer_unternehmen/Zertifizierungsvorgaben_Reis_Reisprodukte_final_160916.pdf