It must be noted that, regarding the following minimum requirements for the scope of analysis, 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. Also, 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 concerning the scope of analysis of raw materials and feeds. In the event that other GMOs become relevant over time (e.g. RASFF reports), VLOG will inform its VLOG-recognised laboratories, members and VLOG-certified companies of any changes in testing 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 testing.

# 1. Minimum requirements for raw materials/single-component feed

## 1.1. 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 and A5547-127:
  In the event of positive result for A2704 and/or A5547-127, the quantity of these GMOs can, for example, be estimated using the ΔΔct method or similar method ensuring that sufficient quantities of species DNA are present. For estimated 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. When using the pat gene (or LibertyLink constructs), A554-127 (or another single copy material) must be used as a reference material for estimation. In the subsequent identification / quantification of positive findings, at least all GMOs (if corresponding elements are positive) mentioned here must be quantified.

## 1.2. Minimum requirements for raw corn/maize materials or corn/maize-based single-component feeds

1. **Screening for 35S Promoter (p35S) and NOS Terminator (tNOS).**
   Other screening elements can be used to narrow down the corresponding GMO.
2. **If positive:**
   In the event of a positive result for 35S Promotor (p35S) and/or NOS Terminator (tNOS), the quantity of these screening elements can, for example, be estimated using the ΔΔct method or a similar method. For estimated values (MON89034 or a suitable reference material for corn/maize that contains 35S in single copy) over 0.1%, identification and subsequent

quantification must be carried out. If there are several positive results, the estimated individual values must be added together.

Testing at least for NK603, TC1507, MON810, MON89034.

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

Positive screening results for values over 0.1% must be clarified; if no GM corn/maize types can be found, other GM types must be analysed, e.g. RRS1.

**4. Determining the summation value of the corn/maize 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 (weight) 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 guideline[1] must take place.

## 1.3. Minimum requirements for raw canola/rapeseed materials / canola/rapeseed-based single-component feeds

There are two possible testing procedures.

**First testing procedure:**

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

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

- tNOS positive: at least RRS + bar gene or MS8 / RF3 directly
- CTP2-CP4epsps / pFMV positive: at least GT73

If no canola/rapeseed GMO is detected, the presence of a botanical contaminant containing GMO with soya or corn must be clarified (estimation and assessment of masses). Is it a relevant quantity or minimal traces? If a botanical contamination containing GMO is determinded, an assessment according to official guidelines[1] must take place.

[1] Guideline for the Control of GMOs in feed (German: Leitfaden zur Kontrolle von GVO in Tierfutter – November 2011 version). Monitoring of the production, of handling, of use and of bringing to market of feed in connection with genetically modified organisms (GMOs). … Developed by the GMOs in Feed Project Group (PG GVO) of the Agricultural Employers Association (LAV) Working Group on Feed, with the participation of the Federal Government and The Association of German Agricultural Investigation and Research Institutions (VDLUFA), https://www.ohnegentechnik.org/fileadmin/ohne-gentechnik/das_siegel/og-standard_english/BVL-Guideline_for_Monitoring_GMOs_in_Feed_180301.pdf

**Second testing procedure:**

1. **Estimating the soy mass:**
   For quantities over 0.**9**%, the quantity of soy GM must be determined (cf. Minimum requirements for feed containing soy).
2. **Qualitative evidence of canola GT73 + canola MS8 or canola RF3 (or bar gene)**
3. **Determining the summation value of GM canola/rapeseed**
   Identified GM canola/rapeseed 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.

## 1.4. Minimum requirements for rice and rice products

1. **Preparation of laboratory samples:**
   Two subsamples of at least 250 g each are to be created from the laboratory sample sent, and each is to be analysed separately (1 extraction, 2 PCRs per subsample:).

2. **Element-specific screening:**
   p35S + tNOS + cry1Ab/cry1Ac sequence

3. **Design-specific proof**
   Version 19.01 Identification, by agreement between the company and the laboratory, of GMO events that cause a positive screening result (see 1).

4. **Exclusion of botanical impurities (GMO carryovers from other plant species) from corn/maize, soy, cotton and (naturally occurring) Cauliflower Mosaic Virus.**

   If the element-specific screening yields a positive result, design-specific proof is to be provided as the next step. In combination with the exclusion of botanical impurities and the Cauliflower Mosaic Virus, it must be investigated whether the sample contains genetically modified rice.

5. **Evaluation of the PCR results**
   If the targeted sequence of genetically modified rice is proven for at least one of the subsamples analysed, this result is to apply to the entire sample and therefore the batch. The batch cannot be marketed in the EU and cannot be labelled with the “Ohne GenTechnik” seal.

## 1.5. Requirements for salmon and salmon products

1. **Design-specific proof**
   AquAdvantage® Atlantic salmon (Salmo salar).
2. **Evaluation of the PCR results:**
   If the targeted sequence of genetically modified salmon is proven for at least one of the subsamples analysed, this result is to apply to the entire sample and therefore the batch. The batch cannot be marketed in the EU and cannot be labelled with the “Ohne GenTechnik” seal.

### 1.6. Requirements for Honey

1. **Detection Method**
   The detection of GM-DNA in honey must be carried out according to § 64 "Amtliche Sammlung von Untersuchungsverfahren" of the German Food and Feed Code (Lebensmittel- und Futtermittelgesetzbuch, LFGB) or in accordance with the BVL guidelines "Guideline on sampling and analysis for the detection of pollen from genetically modified plants in honey".
2. **Preparation of laboratory samples**

   From the submitted laboratory sample (**200 g**), two random samples of at least 50 g are generated and analysed for DNA extraction. | added
3. **Element-specific screening**
   p35S+tNOS+BAR+CTP2-CP4EPSPS+pat sequence or p35S+tNOS+AgroBorderII or comparable screenings
4. **Construct-specific sceening**
   Positive screening results must be confirmed by the detection of at least one GM organism.
5. **Evaluation of the PCR results**
   If the respective target sequence of genetically modified pollen is detected in the analysed sample, this result applies to the entire sample and thus lot. Labelling with the "Ohne GenTechnik" seal is excluded.

## 2. Minimum requirements for compound feed

### 2.1. Minimum requirements for compound feed containing soy

**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 and A5547-127:
  In the event of a positive result for A2704 and A5547-127, 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. Quantification must be carried out for values over 0.1%,.

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

**For corn/maize ingredient:**

Additional qualitative detection of the 4 commercialised corn/maize varieties: NK603, TC1507, MON810, MON89034

In case of a 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. Regular quantification of the GMOs detected must be carried out for values over 0.1%.

In case of limited analysability of the corn/maize ingredient, the limit of detection (LOD) must be indicated.

**For canola/rapeseed 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/rapeseed 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/rapeseed, corn/maize). In the subsequent identification / quantification of positive results, at least all GMOs (if corresponding elements are positive) mentioned here must be identified and, if necessary, quantified.

## 2.2. 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 proportion of GM soy must be determined (cf. Minimum requirements for feed containing soy) and an assessment according to the official guideline[2] must take place.

**For canola/rapeseed 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 case of limited analysability of the canola/rapeseed ingredient, the practical LOD must be indicated.

**For corn/maize ingredient:**

Qualitative evidence of 4 corn/maize varieties used commercially: NK603, TC1507, MON810, MON89034

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 case of limited analysability of the corn/maize ingredient, the practical LOD must be indicated.

[2] Guideline for the Control of GMOs in feed (German: Leitfaden zur Kontrolle von GVO in Tierfutter – November 2011 version). Monitoring of the production, of handling, of use and of bringing to market of feed in connection with genetically modified organisms (GMOs). … Developed by the GMOs in Feed Project Group (PG GVO) of the Agricultural Employers Association (LAV) Working Group on Feed, with the participation of the Federal Government and The Association of German Agricultural Investigation and Research Institutions (VDLUFA), http://www.ohnegentechnik.org/Leitfaden_Futtermittel

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

## 2.3. 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.