

VLOG Carryover Test for Grinding and Mixing Facilities

Guideline for Planning, Implementation and Documentation

Carryover tests are mandatory for grinding and mixing facilities according to VLOG Standard 20.01, Chapter C 6.2, which also lists further information and requirements.

1. Definitions

Test batch: Batch with marker

Follow-up batch: The batch that is checked for carryover. It follows the test batch (after a system purge, if necessary)

System purge: A follow-up batch for “cleansing”, which is run after the test batch to reduce the carryover from the test batch in the follow-up batch

Marker (substance): The means of identifying the test batch in the follow-up batch (e.g. a specific DNA, GMO etc.)

2. Objective of the Carryover Test

The purpose of the carryover test is to determine the carryover quantity from the previous batch in the follow-up batch at a grinding and mixing facility.

3. Purpose of the Carryover Test

The test can validate existing operating procedures or identify problem areas. Depending on the result of the test, measures may have to be taken to reduce carryover in order to comply with GMO threshold values in feed under European legislation¹ and the VLOG Standard.

4. Material used for the Test

The test batch and follow-up batch consist of single-component feeds commonly used in the facility.

If a system purge is conducted, it must comprise material that is typically used for system purges at the facility. It must be ensured (e.g. based on analysis) that the follow-up batch or system purge contain no marker substance or that the precise quantity of the marker substance is known and very low.

5. Processes checked by the Carryover Test

A carryover test must either be conducted for all operating processes performed by the facility (e.g. grinding, crushing or mixing) OR cover the mixing/grinding combination with the greatest risk of carryover used by the grinding and mixing facility. In the second case, the carryover test must comprise test batches and

¹ Regulations EC 1829/2003 and 1830/2003 are most relevant in this context. They specify that GMO content exceeding 0.9% must be labelled as genetically modified in all cases. Values between 0.1% and 0.9% require no labelling only if they are accidental and technically unavoidable (i.e. all operational measures to avoid them were exhausted). Values below 0.1% are generally regarded as accidental and technically unavoidable.

subsequently, follow-up batches run in all work equipment of the facility (e.g. mills, mixers and screw conveyors).

6. Identifying the Carryover

If the follow-up batch contains components of the test batch (carryover), they must be identifiable by the method selected. The marker substance and the detection method must be capable of identifying carryover of 0.1% or less.

Examples of suitable procedures/markers:

- **2 different (types) of pure single-component feed** are used: one for the test batch and the other for the follow-up batch (and the system purge, if relevant)². The carryover of the first type is then determined in the follow-up batch³.
- **GMO as a marker**: a GMO single-component feed is used as the test batch, while a non-GMO feed serves as the follow-up batch⁴. The GMO content in the follow-up batch is determined by PCR analysis to calculate the carryover.

7. Procedure for the Carryover Test

For facilities that operate with complete discharges but not (always) with system purges in VLOG production

- 1) Process the test batch as usual
- 2) Perform a normal complete discharge
- 3) Process the follow-up batch as usual
- 4) Take a representative sample of the follow-up batch
- 5) Analyse the sample and evaluate the results

For facilities that operate with system purges but not (always) with complete discharges in VLOG production

- 1) Process the test batch as usual
- 2) Perform a system purge with the usual minimum quantity
- 3) Process the follow-up batch as usual
- 4) Take a representative sample of the follow-up batch
- 5) Analyse the sample and evaluate the results

² See Example A), below

³ The species that can be identified with sufficient precision must be agreed upon with the laboratory (e.g. identified by PCR analysis, as microscopic methods generally don't offer satisfactory results in this context). We recommend using soy for the test batch and having a VLOG-recognised laboratory determine the percentage of soy in the follow-up batch.

⁴ A sufficiently large GMO content in the test batch is necessary for meaningful results (min. 30% GMO). Please note that "GMO feed" normally does not contain 100% GMO, but, at times, significantly less. In this scenario, a VLOG-recognised laboratory must analyse both the test batch and the system purge and follow-up batch for GMOs. See Example B), below.

For facilities that always operate with complete discharges and system purges in VLOG production⁵

- 1) Process the test batch as usual
- 2) Perform a system purge with the usual minimum quantity (e.g. in accordance with manufacturer specifications)
- 3) Perform a normal complete discharge
- 4) Process the follow-up batch as usual
- 5) Take a representative sample of the follow-up batch
- 6) Analyse the sample and evaluate the results

8. Representative Sampling

Since most carryover does not occur evenly during the mixing process, measures must be taken to ensure that the sample is representative. There are two options:

- A) Take samples of the same size (min. 400 g each) from the batch at several evenly distributed time intervals. In this process, take one sample at the start and one at the very end⁶. Mix the individual samples well (e.g. in a clean bucket) and take a final sample (min. 400 g) from this aggregate sample.
- B) Thoroughly remix the entire follow-up batch after passing through the system (e.g. after it has again been taken up by the system) and then take the sample (min. 400 g).

Place the sample into a suitable container (e.g. a bag) with a clean tool, clearly label the container (e.g. with date and number) and apply a tamper-proof seal.

Note: It is always advisable to take a second sample of the same size and keep it as a reference sample in case another test is required/needed later.

9. Analysis of the Follow-Up Batch and Carryover Calculation

The follow-up batch is analysed for carryover from the test batch to determine the test batch proportion still present in the follow-up batch (based on the identification procedure). To this end, the amount in the follow-up batch is multiplied by the percentage of carryover material.

Example: The follow-up batch weighs 2,000 kg. The carryover quantity from the test batch in the follow-up batch is 0.3% → $2,000 \times 0.3\% = 6$ kg of carryover material

10. Evaluation of the Results and Determination of Measures

If the results show that the current operational measures taken at the facility are not sufficient for maximum reduction of carryover (see Section 3.), facility-specific measures must be developed to reduce the carryover to an acceptable level. Such measures may include:

- Introducing a system purge/complete discharge

⁵Carryover tests are not mandatory in such facilities under normal operating conditions according to the VLOG Standard. However, the test may serve as a safeguard in such facilities.

⁶ It is advisable to take at least 5 samples from the mixture. The larger the batch, the more samples should be taken. The number of samples has to verify that the final sample represents the whole batch and its state of mixture. The consistent distribution of the marker (substance) has to be assured.

- Expanding the system purge
- Specifying the maximum GMO content in the mixture that is run before the VLOG mixture
- Changing the scheduling/sequence of processing (e.g. previous application only involves crushing; no GMO content in the previous mixture)
- Etc.

11. Documentation of the Carryover Test

The carryover test must be clearly documented. This includes in particular:

- System (model, registration no.), tester, date
- Processes tested (e.g. mixing, grinding, screw conveyor...)
- Description of test batch, follow-up batch and system purge, if any (type, quantity)
- Description of marker (type and quantity of micro-tracers, various single-component feeds, GMOs, ...)
- Description of measures (e.g. system purge, complete discharge)
- Test procedure (including variants, if any)
- Analytical method, markers used
- Laboratory/test reports, etc. must be retained
- Results of the carryover test
- Any (corrective) measures taken/developed

12. Specific Examples for the Carryover Test

Example A: Conducting the test with two different single-component feeds

A pure single-component feed – in this case, soy – is used as a test batch. This is followed by the usual cleansing procedure – in this case, a complete discharge. In a next step, the follow-up batch is run through the system with another single-component feed having no soy component – in this case, rye. A representative sample is taken from this follow-up batch, and the sample is checked for soy components.

- 1) Test batch: 1,000 kg of soy are ground and mixed
- 2) A normal complete discharge is performed
- 3) Follow-up batch: 1,000 kg of rye are ground and mixed
- 4) Sampling: An aggregate sample, formed from the individual samples, is mixed well to take a final individual sample for analysis.
- 5) The soy content of the follow-up batch is checked (e.g. with PCR analysis)
- 6) The carryover is calculated based on the amounts used in the test batch and the follow-up batch.

Example of measures taken based on the results:

Test result: The carryover after complete discharge is 0.5%. In a 1,000 kg follow-up batch, this corresponds to 5 kg (0.5% multiplied by 1,000 kg= 5 kg).

Objective: GMO carryover must be reduced to below 0.1% if technically feasible (see European legislation in Section 3.).

Possible measures:

- 1,000-kg VLOG mixtures can only be preceded by GMO mixtures containing less than 20% of material subject to compulsory labelling⁷
- In addition to a complete discharge, a system purge is performed

Example B: Conducting the test with a GMO as the marker

A single-component GMO feed – in this case, GMO soy meal – is used as a test batch, followed by a normal complete discharge and a system purge with non-GMO wheat. In a next step, a follow-up batch is run with non-GMO material – in this case, wheat again. A representative sample is taken from the follow-up batch, and the sample is checked for GMOs.

- 1) Test batch: 2,000 kg of GMO soy meal are ground and mixed
- 2) The GMO soy meal is analysed for GMOs after running through the system (result, e.g., 50% GMOs)
- 3) A normal complete discharge is performed
- 4) The wheat for the system purge and follow-up batch is analysed for GMOs in advance (result, e.g., <0.1% GMO soy content in the wheat)⁸
- 5) A normal system purge is performed in accordance with manufacturer recommendations, using 300 kg of wheat
- 6) Follow-up batch: 1,000 kg of wheat are ground and mixed (follow-up batch)
- 7) The follow-up batch is analysed for GMO soy
- 8) The carryover is calculated based on the GMO content of the soy meal and the amounts used in the test batch and the follow-up batch

⁷ Method of calculation: 0.5% is five times the target value of 0.1%. Therefore, if the GMO content is reduced to less than one fifth (<20%), the carryover is brought below 0.1%

⁸ Analysis of the wheat may be omitted if contamination with GMO can be ruled out (e.g., if the wheat was cultivated in Germany and there was no processing, transport or the like during which the wheat could have become contaminated with GMOs).