

DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

25/2014

GMO - Screening qualitative:

**5 Samples with positive/negative
amounts of other GMOs in Papaya,
Rape and Honey**

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Content

| | |
|--|----|
| 1. Introduction..... | 3 |
| 2. Realisation..... | 3 |
| 2.1 Test material..... | 3 |
| 2.2 Test..... | 3 |
| 2.3 Submission of results..... | 3 |
| 3. Evaluation..... | 5 |
| 4. Results..... | 5 |
| 4.1 Test..... | 6 |
| 4.1.1 Results: 35S-Screening-Sequence..... | 6 |
| 4.1.2 Results: NOS-Screening-Sequence..... | 7 |
| 4.1.3 Results: Papaya-specific DNA..... | 8 |
| 4.1.4 Results: GMO-Papaya..... | 9 |
| 4.1.5 Results: Rape-specific DNA..... | 10 |
| 4.1.6 Results: GMO Rape..... | 11 |
| 4.1.7 Results: Other Parameters (DNA)..... | 12 |
| 5. Documentation..... | 13 |
| 5.1 Details by participants about DNA-Extraction methods.... | 13 |
| 5.1.1 35S-Screening Sequence..... | 13 |
| 5.1.2 NOS-Screening Sequence..... | 13 |
| 5.1.3 Papaya specific DNA..... | 14 |
| 5.1.4 GMO Papaya..... | 14 |
| 5.1.5 Rape-specific DNA..... | 15 |
| 5.1.6 GMO Rape..... | 15 |
| 5.1.7 Other Parameters (DNA)..... | 16 |
| 5.2 Details by participants to PCR-reaction..... | 17 |
| 5.2.1 35S-Screening Sequence..... | 17 |
| 5.2.2 NOS-Screening Sequence..... | 17 |
| 5.2.3 Papaya-specific DNA..... | 18 |
| 5.2.4 GMO Papaya..... | 18 |
| 5.2.5 Rape-specific DNA..... | 19 |
| 5.2.6 GMO Rape..... | 19 |
| 5.2.7 Other Parameters (DNA)..... | 20 |
| 6. Index of participant laboratories..... | 21 |
| 7. Index of references..... | 22 |

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the validity of the particular testing method.

The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.

Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009.

2. Realisation

2.1 Test material

The test materials are 5 different mixtures of common in commerce foods from European and non-EU suppliers (s. table 1).

The ingredients were mixed, homogenized and portioned to approximately 10 g.

The materials were tested for homogeneity.

2.2 Test

One portion of each of the 5 test materials was sent to every participating laboratory in the 28th week of 2014. The testing method was optional. The tests should be finished at August 22nd 2014 the latest.

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out along with the samples. The results given as positive/negative were evaluated with respect to each tested parameter. Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.

All participants submitted their results in time.

Table 1: Composition of DLA-samples

| DLA-Sample | Ingredients (per 100 g) | GMO-Con-tent |
|-------------------|--|---|
| 1 | Papaya-Mixture (100 g) Ingredients: Potato flour, papaya dried (33%), sugar | - |
| 2 | Candied Papaya-Mixture (100 g) Ingredients: Potato flour, papaya candied with papaya, sugar and preservative E220 (34%), maize flour | positive (35S or NOS, experimental) |
| 3 | Rape Oilcake, European Supplier (100 g) Ingredients: Rape oilcake | positive (35S or NOS, experimental) |
| 4 | Maize Flour, European-Supplier (85 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 77 g, Fat 1 g | - |
| 5 | Honey Mixture, EU- and Non-EU Countries (100 g) Ingredients: several Honeys | positive (35S or NOS, experimental) |

3. Evaluation

The evaluation of the GMO-screening proficiency test was done exclusively qualitative.

The results are presented for all 5 test samples in separate tables for each parameter 35S, NOS, Papaya-DNA and Rape-DNA and other DNA results. The numbers and percentage of positive and negative results are given at the end of each table. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample.

For every participant a qualitative valuation is made with respect to the consensus results. Therefore the number and percentage of "correct" results of consensus results is given.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

The results of the participants are given in tables as indicated below:

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 4 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|-----------------------------------|---------|
| Parameter | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus results | |

4.1 Test

4.1.1 Results: 35S-Screening-Sequence

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|---------------------------------|---|
| 35S | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus value | |
| 1 | negative | positive | positive | positive | negative | 3/4 (75%) | |
| 2 | negative | positive | positive | positive | positive | 3/4 (75%) | |
| 3 | negative | positive | positive | negative | positive | 4/4 (100%) | |
| 4 | negative | positive | positive | negative | negative | 4/4 (100%) | |
| 5 | negative | positive | positive | negative | positive | 4/4 (100%) | Sample 3: weakly positive ct 35,5; Sample 5: weakly positive ct 36,9 |
| 6 | negative | positive | positive | negative | negative | 4/4 (100%) | Sample 5: traces |
| 7 | | | | | positive | | |
| 8 | negative | positive | positive | negative | positive | 4/4 (100%) | |
| 9 | negative | positive | positive | negative | negative | 4/4 (100%) | Sample 2: 8,35%, Sample 3: 40,09% |
| 10 | negative | positive | negative | negative | negative | 3/4 (75%) | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------|----------|----------|----------|----------|----------|
| Number positive | 0 | 9 | 8 | 2 | 5 |
| Number negative | 9 | 0 | 1 | 7 | 5 |
| Percent positive | 0 | 100 | 89 | 22 | 50 |
| Percent negative | 100 | 0 | 11 | 78 | 50 |
| Consensus | negative | positive | positive | negative | none |

Comments on results:

There were consensus values obtained for 4 samples with two times 100%, 89% and 78% positive or negative results. For sample 5 (honey) one half of results were positive and the other negative.

4.1.2 Results: NOS-Screening-Sequence

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|---------------------------------|---|
| NOS | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus value | |
| 1 | negative | positive | positive | positive | negative | 3/4 (75%) | |
| 2 | negative | positive | positive | positive | negative | 3/4 (75%) | |
| 3 | negative | positive | positive | negative | positive | 4/4 (100%) | |
| 4 | negative | positive | positive | negative | negative | 4/4 (100%) | |
| 5 | negative | positive | positive | negative | positive | 4/4 (100%) | Sample 3: weakly positive ct 35,6; Sample 5: weakly positive ct 37,0 |
| 6 | negative | positive | positive | negative | negative | 4/4 (100%) | |
| 7 | | | | | positive | | |
| 8 | negative | positive | positive | negative | positive | 4/4 (100%) | |
| 9 | negative | positive | positive | negative | negative | 4/4 (100%) | Sample 2: 6,48%, Sample 3: 46,44% |
| 10 | negative | positive | negative | negative | negative | 3/4 (75%) | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------|----------|----------|----------|----------|----------|
| Number positive | 0 | 9 | 8 | 2 | 4 |
| Number negative | 9 | 0 | 1 | 7 | 6 |
| Percent positive | 0 | 100 | 89 | 22 | 40 |
| Percent negative | 100 | 0 | 11 | 78 | 60 |
| Consensus | negative | positive | positive | negative | none |

Comments on results:

There were consensus values obtained for 4 samples with two times 100%, 89% and 78% positive or negative results. For sample 5 (honey) about one half of results were positive and the other negative.

4.1.3 Results: Papaya-specific DNA

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|---------------------------------|---------|
| Papaya | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus value | |
| 1 | | | | | | - | |
| 2 | | | | | | - | |
| 3 | positive | negative | negative | negative | | - | |
| 4 | | | | | | - | |
| 5 | | | | | | - | |
| 6 | positive | negative | negative | negative | negative | - | |
| 7 | | | | | | - | |
| 8 | | | | | | - | |
| 9 | positive | negative | negative | negative | negative | - | |
| 10 | | | | | | - | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------|----------|----------|----------|----------|----------|
| Number positive | 3 | 0 | 0 | 0 | 0 |
| Number negative | 0 | 3 | 3 | 3 | 2 |
| Percent positive | 100 | 0 | 0 | 0 | 0 |
| Percent negative | 0 | 100 | 100 | 100 | 100 |
| Consensus | - | - | - | - | - |

Comments on results:

Only three participants tested the samples for specific papaya-DNA. Therefore no consensus values were set. In sample 1 (containing dried papaya) all three participants detected papaya. In sample 2 candied papaya fruit was contained, which could not be detected by the participants. In samples 3, 4 and 5 no papaya was added.

4.1.4 Results: GMO-Papaya

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|---------------------------------|---------|
| GMO-Papaya | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus value | |
| 1 | | | | | | - | |
| 2 | | | | | | - | |
| 3 | negative | | | | | - | |
| 4 | | | | | | - | |
| 5 | | | | | | - | |
| 6 | negative | negative | negative | negative | | - | |
| 7 | | | | | | - | |
| 8 | | | | | | - | |
| 9 | negative | | | | | - | |
| 10 | | | | | | - | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------|----------|----------|----------|----------|----------|
| Number positive | 0 | 0 | 0 | 0 | 0 |
| Number negative | 3 | 1 | 1 | 1 | 0 |
| Percent positive | 0 | 0 | 0 | 0 | 0 |
| Percent negative | 100 | 100 | 100 | 100 | 0 |
| Consensus | - | - | - | - | - |

Comments on results:

Only three participants tested the samples for specific papaya-DNA. Therefore no consensus values were set. There were exclusively negative results.

4.1.5 Results: Rape-specific DNA

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|---------------------------------|------------------|
| Rape DNA | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus value | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | negative | negative | positive | negative | positive | 2/2 (100%) | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | negative | negative | positive | negative | positive | 2/2 (100%) | Sample 1: traces |
| 7 | | | | | | | |
| 8 | | positive | negative | | negative | 0/2 (0%) | |
| 9 | negative | negative | positive | negative | negative | 2/2 (100%) | |
| 10 | | | | | | | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------|----------|----------|----------|----------|----------|
| Number positive | 0 | 1 | 3 | 0 | 2 |
| Number negative | 3 | 3 | 1 | 3 | 2 |
| Percent positive | 0 | 25 | 75 | 0 | 50 |
| Percent negative | 100 | 75 | 25 | 100 | 50 |
| Consensus | - | negative | positive | - | none |

Comments on results:

Only four participants tested the samples for specific rape-DNA. Consensus values were obtained for samples 2 and 3, for which four results and 75% negative and positive results were submitted. The consensus values are in agreement with the addition of rape to samples 2 (rape not added) and 3 (added).

4.1.6 Results: GMO Rape

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Qualitative Valuation | Remarks |
|-------------------|----------|----------|----------|----------|----------|---------------------------------|---------|
| GMO-Rape | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg | Agreements with consensus value | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | negativ | negativ | positive | negativ | positive | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | negativ | negativ | negativ | negativ | negativ | | |
| 7 | | | | | | | |
| 8 | | positive | negativ | | negativ | | |
| 9 | | | | | | | |
| 10 | | | | | | | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------|----------|----------|----------|----------|----------|
| Number positive | 0 | 1 | 1 | 0 | 1 |
| Number negativ | 2 | 2 | 2 | 2 | 2 |
| Percent positive | 0 | 33 | 33 | 0 | 33 |
| Percent negativ | 100 | 67 | 67 | 100 | 67 |
| Consensus | - | - | - | - | - |

Comments on results:

Only three participants tested the samples for GMO-rape. Therefore no consensus values were set.

4.1.7 Results: Other Parameters (DNA)

| Evaluation number | Parameter | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|-------------------|----------------|------------|------------|------------|------------|---------------------|
| | Other DNA | pos/neg | pos/neg | pos/neg | pos/neg | pos/neg |
| 1 | FMV | negative | negative | negative | negative | negative |
| 3a | bar | negative | negative | negative | negative | negative |
| 3b | pat | negative | positive | negative | negative | negative |
| 3c | CTP2-CP4 EPSPS | negative | positive | negative | negative | negative |
| 3d | Maize specific | negative | positive | positive | positive | negative |
| 3e | Maize GVO | negative | positive | | negative | negative |
| 4a | FMV | negative | negative | negative | negative | negative |
| 4b | RR-Soya | negative | positive | positive | negative | negative |
| 5 | FMV | negative | negative | negative | negative | negative |
| 7a | MON 40-3-2 | | | | | positive |
| 7b | Maize und Raps | | | | | positive |
| 8a | Maize specific | n.d. | positive | negative | n.d. | negative |
| 8b | Maize MON810 | n.d. | positive | negative | n.d. | negative |
| 8c | Maize LLT25 | n.d. | positive | negative | n.d. | negative |
| 8d | Soya specific | n.d. | negative | positive | n.d. | schwach positive |
| 8e | Soya RRS I | n.d. | negative | positive | n.d. | schwach positive |
| 8f | trans-EPSPS | negative | positive | negative | negative | negative |
| 8g | syn-PAT | negative | positive | negative | negative | negative |
| 8h | bar | negative | negative | negative | negative | negative |
| 8i | SAMS-HRA | negative | negative | negative | negative | negative |
| 8j | DNA-extraction | NucleoSpin | NucleoSpin | NucleoSpin | NucleoSpin | CTAB |
| 9a | Mir604 | - | 0,10% | negative | - | - |
| 9b | TC1507 | - | 0,20% | negative | - | - |
| 9c | Mon88017 | - | 5,80% | negative | - | - |
| 9d | Mon810 | - | 4,10% | negative | - | - |
| 9e | NK603 | - | 4,10% | negative | - | - |
| 9f | GA21 | - | 0,01% | negative | - | - |
| 9g | Mon863 | - | 0,40% | negative | - | - |
| 9h | T25 | - | 0,30% | negative | - | - |
| 9i | 59122 | - | 4,10% | negative | - | - |
| 9j | 98140 | - | negative | positive | - | - |
| 9k | Pat | negative | 0,15% | negative | negative | negative |
| 9l | EPSPS | negative | 0,07% | negative | negative | negative |
| 9m | Bar | negative | negative | negative | negative | negative |
| 9n | FMV | negative | negative | negative | negative | negative |
| 9o | Roundup ready | - | - | 51,40% | - | |
| 9p | Soya specific | negative | negative | positive | negative | |
| 9q | Maize specific | negative | positive | positive | positive | |
| 10a | FMV | negative | negative | negative | negative | negative |
| 10b | Bar | negative | negative | negative | negative | negative |

5. Documentation

5.1 Details by participants about DNA-Extraction methods

5.1.1 35S-Screening Sequence

| Evaluation number | Result given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|-------------------|-----------------------|---------------------------------------|--|
| | Target-Sequenz / -DNA | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| 1 | 35S | R-Biopharm | according to manual, SureFood Prep Plant X, Art. No. S1006 |
| 2 | | | |
| 3 | | ASU (§64 LFGB) L 00.00-122 | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| 4 | | r-biopharm | FFS-Kit (Promega) |
| 5 | 35S | Congen 35S/nos/FMV Monoplex | Congen Prep PLANT Extraction kit |
| 6 | 35S-Promotor | DIN EN ISO 21570:20096 | Macherey and Nagel Nucleospin |
| 7 | | | |
| 8 | | | |
| 9 | | AIIGVOSC B, Köppel | Promega Wizard |
| 10 | | Biotecon, foodproof GMO Screening Kit | Surefood GMO Plant and Plant X |

5.1.2 NOS-Screening Sequence

| Evaluation number | Result given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|-------------------|-----------------------|---------------------------------------|--|
| | Target-Sequenz / -DNA | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| 1 | NOS | R-Biopharm | according to manual, SureFood Prep Plant X, Art. No. S1006 |
| 2 | | | |
| 3 | | ASU (§64 LFGB) L 00.00-122 | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| 4 | | r-biopharm | FFS-Kit (Promega) |
| 5 | nos | Congen 35S/nos/FMV Monoplex | Congen Prep PLANT Extraction kit |
| 6 | NOS-Terminator | DIN EN ISO 21570:20096 | Macherey and Nagel Nucleospin |
| 7 | | | |
| 8 | | | |
| 9 | | AIIGVOSC B, Köppel | Promega Wizard |
| 10 | | Biotecon, foodproof GMO Screening Kit | Surefood GMO Plant and Plant X |

5.1.3 *Papaya specific DNA*

| Evaluation number | Result given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|-------------------|-----------------------|--|--|
| | Target-Sequenz / -DNA | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| 1 | | | |
| 2 | | | |
| 3 | Papain | Xu et al (2008) Eur Food Res Technol 228: 301-309. | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| 4 | | | |
| 5 | | | |
| 6 | Papain-Gene | LFGB L29:00-9 | |
| 7 | | | |
| 8 | | | |
| 9 | Papain | Wall et al., 2004 | Promega Wizard |
| 10 | | | |

5.1.4 *GMO Papaya*

| Evaluation number | Result given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|-------------------|-----------------------|------------------------|--|
| | Target-Sequenz / -DNA | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| 1 | | | |
| 2 | | | |
| 3 | | | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | 55-1 | Wall et al., 2004 | Promega Wizard |
| 10 | | | |

5.1.5 Rape-specific DNA

| Evaluation number | Result given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|-------------------|-----------------------|--|--|
| | Target-Sequenz / -DNA | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| 1 | | | |
| 2 | | | |
| 3 | PEP | Methods according to § 28 GenTG: Method G 30.40-1 | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| 4 | | | |
| 5 | | | |
| 6 | cruziferin A-Gene | CRLVL26/04VP | |
| 7 | | | |
| 8 | | | |
| 9 | Cruciferin A | Laube et al., 2008 | Promega Wizard |
| 10 | | | |

5.1.6 GMO Rape

| Evaluation number | Result given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|-------------------|-----------------------|------------------------|--|
| | Target-Sequenz / -DNA | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| 1 | | | |
| 2 | | | |
| 3 | | | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | Rape GT73 | | |
| 9 | | | |
| 10 | | | |

5.1.7 Other Parameters (DNA)

| Parameter | Evaluation number | Results given as | Test-Kit or Literature | Remarks to DNA-Extraction |
|----------------|-------------------|------------------|---|---|
| | | Target-Sequence | Supplier / Method | e.g. Extraction / Enzymes / Clean-Up / DNA-Quality |
| other | 1 | FMV | R-Biopharm | according to manual, SureFood Prep Plant X, Art. Nr. S1006 |
| other | 3a | bar | ASU (§64 LFGB) L 00.00-154 (08/2014) | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| other | 3b | pat | ASU (§64 LFGB) L 00.00-154 (08/2014) | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| other | 3c | CTP2-CP4 EPSPS | ASU (§64 LFGB) L 00.00-154 (08/2014) | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| Maize specific | 3d | hmg | Hernandez M. et al (2004) J. Agric. Food Chem. 52:4632-4637 | CTAB/QIAquick Purification Kit for samples 1-4; sample 5 honey : ASU L 40.00-14 |
| Maize GVO | 3e | | | |
| FMV | 4a | | r-biopharm | FFS-Kit (Promega) |
| RR-Soya | 4b | | r-biopharm | FFS-Kit (Promega) |
| other | 5 | FMV | Congen 35S/nos/FMV Monoplex | Congen Prep PLANT Extraction kit |
| other | 7a | MON 40-3-2 | | |
| other | 7b | Maize and Rape | | |
| Maize specific | 8a | | | |
| Maize MON810 | 8b | | | |
| Maize LLT25 | 8c | | | |
| Soya specific | 8d | | | |
| Soya RRS I | 8e | | | |
| trans-EPSPS | 8f | | | |
| syn-PAT | 8g | | | |
| bar | 8h | | | |
| SAMS-HRA | 8i | | | |
| DNA-Extraktion | 8j | | | |
| Mir604 | 9a | | AllMaizeF, Köppel | Promega Wizard |
| TC1507 | 9b | | AllMaizeF, Köppel | Promega Wizard |
| Mon88017 | 9c | | AllMaizeE, Köppel | Promega Wizard |
| Mon810 | 9d | | AllMaizeC, Köppel | Promega Wizard |
| NK603 | 9e | | AllMaizeD, Köppel | Promega Wizard |
| GA21 | 9f | | AllMaizeE, Köppel | Promega Wizard |
| Mon863 | 9g | | AllMaizeC, Köppel | Promega Wizard |
| T25 | 9h | | AllMaizeC, Köppel | Promega Wizard |
| 59122 | 9i | | AllMaizeE, Köppel | Promega Wizard |
| 98140 | 9j | | AllMaizeF, Köppel | Promega Wizard |
| Pat | 9k | | AllGVOSc C, Köppel | Promega Wizard |
| EPSPS | 9l | | AllGVOSc C, Köppel | Promega Wizard |
| Bar | 9m | | AllGVOSc C, Köppel | Promega Wizard |
| FMV | 9n | | AllGVOSc C, Köppel | Promega Wizard |
| Roundup ready | 9o | | AllSoyA, Köppel | Promega Wizard |
| Soya specific | 9p | Lectin | AllGVOSc B, Köppel | Promega Wizard |
| Maize specific | 9q | mhmg | AllGVOSc B, Köppel | Promega Wizard |
| FMV | 10a | FMV | Biotecon, foodproof GMO Screening Kit | Surefood GMO Plant und Plant X |
| Bar | 10b | Bar | Biotecon, foodproof GMO Screening Kit | Surefood GMO Plant und Plant X |

5.2 Details by participants to PCR-reaction

5.2.1 35S-Screening Sequence

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|---|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | Real Time PCR, SureFood GVO Screening 4plex, Art. No. S2126 | |
| 2 | | |
| 3 | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 4 | real time PCR, 45 cycles | |
| 5 | Real Time PCR, 45 Cycles, Reference material ERM-BF 410dk | positive*: weakly positive ct 35,5 positive**: weakly positive ct 36,9 |
| 6 | Real Time PCR | |
| 7 | | |
| 8 | RealTime-PCR | |
| 9 | | |
| 10 | Real Time PCR, Stratagene MX3005P, 50Cycles | |

* sample 3

** sample 5

5.2.2 NOS-Screening Sequence

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|---|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | Real Time PCR, SureFood GVO Screening 4plex, Art. No. S2126 | |
| 2 | | |
| 3 | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 4 | real time PCR, 45 cycles | |
| 5 | Real Time PCR, 45 Cycles, Reference material ERM-BF 410dk | positive*: weakly positive ct 35,6 positive**: weakly positive ct 37,0 |
| 6 | Real Time PCR | |
| 7 | | |
| 8 | RealTime-PCR | |
| 9 | | |
| 10 | Real Time PCR, Stratagene MX3005P, 50Cycles | |

* sample 3

** sample 5

5.2.3 *Papaya-specific DNA*

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|-----------------|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | | |
| 2 | | |
| 3 | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |
| 10 | | |

5.2.4 *GMO Papaya*

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|--|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | | |
| 2 | | |
| 3 | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | direct GMO determination was not performed; only screening results were valuated |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |
| 10 | | |

5.2.5 Rape-specific DNA

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|-----------------|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | | |
| 2 | | |
| 3 | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | RealTime-PCR | |
| 9 | | |
| 10 | | |

5.2.6 GMO Rape

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|--|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | | |
| 2 | | |
| 3 | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | direct GMO determination was not performed; only screening results were valuated |
| 4 | | |
| 5 | | |
| 6 | | In sample 3 evtl. GMO-Rape present? (oxy235?) |
| 7 | | |
| 8 | RealTime-PCR | |
| 9 | | |
| 10 | | |

5.2.7 Other Parameters (DNA)

| Evaluation number | Notes to PCR-Reaction | Further Remarks |
|-------------------|---|--|
| | e.g. Real Time PCR / Gel electrophoresis / Cycles / Length of Amplificates / Reference material | |
| 1 | Real Time PCR, SureFood GMO Screening 4plex, Art. No. S2126 | |
| 3a | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 3b | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 3c | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 3d | Real Time PCR each. evaluation by comparison with DNA-Standard on plasmid basis (10 copies). Ct-values of samples, which are about 1 or more above Ct of Standard-DNA, were valuated "negative" | |
| 3e | | direct GMO determination was not performed; only screening results were valuated |
| 4a | real time PCR, 45 cycles | |
| 4b | real time PCR, 45 cycles | |
| 5 | Real Time PCR, 45 cycles, reference material ERM-BF 410dk | |
| 7a | | |
| 7b | | |
| 8a | RealTime-PCR | |
| 8b | RealTime-PCR | |
| 8c | RealTime-PCR | |
| 8d | RealTime-PCR | |
| 8e | RealTime-PCR | |
| 8f | RealTime-PCR | |
| 8g | RealTime-PCR | |
| 8h | RealTime-PCR | |
| 8i | RealTime-PCR | |
| 8j | | |
| 9a | | % GMO related to Soya- or Maize part |
| 9b | | % GMO related to Soya- or Maize part |
| 9c | | % GMO related to Soya- or Maize part |
| 9d | | % GMO related to Soya- or Maize part |
| 9e | | % GMO related to Soya- or Maize part |
| 9f | | % GMO related to Soya- or Maize part |
| 9g | | % GMO related to Soya- or Maize part |
| 9h | | % GMO related to Soya- or Maize part |
| 9i | | % GMO related to Soya- or Maize part |
| 9j | | % GMO related to Soya- or Maize part |
| 9k | | |
| 9l | | |
| 9m | | |
| 9n | | |
| 9o | | % GMO related to Soya- or Maize part |
| 9p | | |
| 9q | | |
| 10a | Real Time PCR, Stratagene MX3005P, 50cycles | |
| 10b | Real Time PCR, Stratagene MX3005P, 50cycles | |

6. Index of participant laboratories

| <u>Teilnehmer / Participant</u> | <u>Ort / Town</u> | <u>Land / Country</u> |
|---------------------------------|-------------------|-----------------------|
| | | THAILAND |
| | | GERMANY |
| | | GERMANY |
| | | GERMANY |
| | | GERMANY |
| | | GERMANY |
| | | SWITZERLAND |
| | | GERMANY |
| | | GERMANY |
| | | GERMANY |

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

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