FEDERAL INSTITUTE FOR RISK ASSESSMENT

Risiken erkennen – Gesundheit schützen

3rd International Workshop on Harmonisation of GMO Detection and Analysis for Central and South America, Cartagena, Colombia, 3 - 4 July 2012

Detection of GMO traces in honey: update on activities and detection strategies

Hermann Broll

Federal Institute for Risk Assessment (BfR)
Berlin, Germany

From „Health Office“ (Gesundheitsamt) to the BfR

- Kaiserliches Gesundheitsamt (1876-1919)
- Reichsgesundheitsamt (1919-45)
-
-
- Bundesgesundheitsamt (1952-1994)
  „Federal Health Office“
- Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (1994-2002)

“Federal Institute for Health protection of Consumers and Veterinary Medicine”

BfR
Risiken erkennen – Gesundheit schützen

risk assessment

Gesetz zur Neuorganisation des gesundheitlichen Verbraucherschutzes und der Lebensmittelsicherheit

Vom 6. August 2002

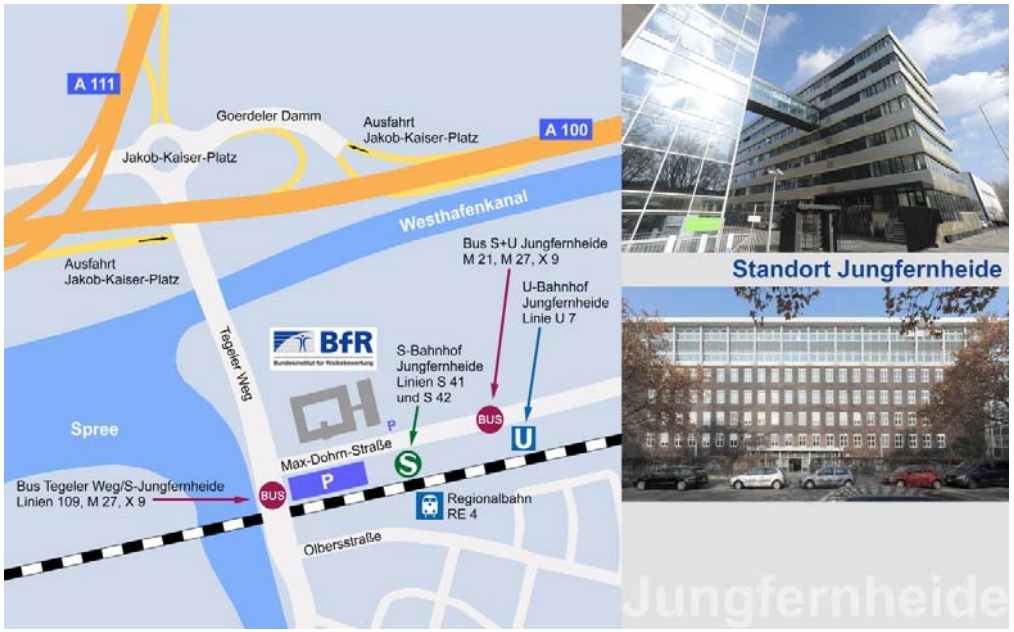
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

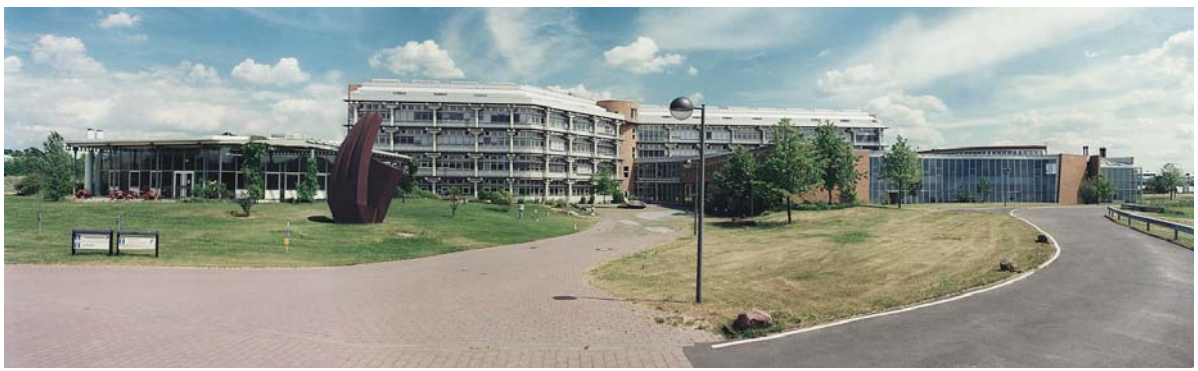
risk management

Locations

The BfR is located in three areas in

- Berlin-Jungfernheide and
- Berlin-Marienfelde





Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR)

- Established 1st of November 2002;
- 31st of October 2002: BgVV disappeared by law
- based on an expert opinion asked by the federal government regarding food safety;
- separation of risk assessment and risk management;
- BfR duties:
  - risk identification in advance,
  - preparation of scientific opinions and reports and
  - Communication of those hazards and risks to the public.

Content

- Honey in the European legal framework after European court decision
- Outcome of an international workshop in Berlin, Germany 13-14 Dec 2011
- Method validation for DNA extraction from pollen
- Strategy for the analysis of the presence of gm pollen
- Recommendations

Honey with gm pollen in the European legal framework after European court decision

European Union situation regarding gm pollen in honey

- √ **In the European Union (EU), food containing ingredients from genetically modified (gm) organisms have to fulfil the legal requirements of regulations (EC) No. 1829/2003 and 1830/2003;**
- √ **The regulations imply that genetically modified (gm) plants used for food production have to be authorised before marketing, and a labelling threshold of 0.9 % has to be applied;**
- √ **So far, the European Commission (EC) was of the opinion that pollen from gm plants in honey is not regarded as an ingredient and therefore not falling under the scope of the above mentioned regulations.**

ISO 17025

European Union situation regarding gm pollen in honey

- **In September 2011, the Court of Justice of the European Union decided that pollen of gm plants in honey ‘‘must be classified as an ‘ingredient’ and the pollen in question consequently falls within the scope of the regulation and must be subject to the authorisation scheme provided for there under before being placed on the market’’;**
- **Consequently, gm plants need a full scope authorisation covering the use of their pollen in honey;**
- **For control purposes, harmonized and validated methods are needed for the detection of pollen from gm plants in honey.**

ISO 17025

Method validation for DNA extraction from pollen

- ✓ **Methods for the analysis of GMO are used and combined in a modular way;**
- ✓ **In honey analysis, for most modules, validated and standardised methods are already available;**
- ✓ **Generally, real-time PCR methods are commonly used for the detection and quantification of gm material in foods;**
- ✓ **Such methods are already validated in collaborative studies and available e.g. as national (e.g. German Official Collection according to §64 LFGB) or international standards (e.g., ISO 21569: 2005);**
- ✓ **The EURL-GMFF is validating real-time PCR methods for the detection of gm plants to be authorised under the scope of Regulation (EC) Nr. 1829/2003;**
- ✓ **However, for the module 'DNA extraction from honey', interlaboratory validated methods were still missing.**

International Workshop on the consequences of the ECJ judgement on GM pollen in honey for GM crop releases and cultivation in Germany and the EU

Berlin, December 13-14, 2011

Federal Ministry
of Food, Agriculture
and Consumer Protection

International Workshop

on the consequences of the ECJ judgement on GM pollen in honey for GM crop releases and cultivation in Germany and the EU

Berlin, December 13-14, 2011

Working group report (WG2)

Detection methods for GMP pollen in honey

- App. 50 participants from all over the world took part in the discussion

1. Open discussion on general issues:

- **Contribution from Argentina about questioning related to the analytical procedures:**
- · **Pollen coming from different botanical sources**
- · **Harmonisation from one lab to another**
- · **Sensitivity of the PCR method**
- **Relationship between official control and private laboratories testing for companies:**

validated method vs. *in-house* methods

need for international standardisation and harmonisation

need for Proficiency testing schemes

International Workshop

on the consequences of the ECJ judgement on GM pollen in honey for GM crop releases and cultivation in Germany and the EU

Berlin, December 13-14, 2011

Working group report (WG2)

Detection methods for GMP pollen in honey

Detailed discussion on analytical steps:

- **Sampling**
- **Pollen isolation from honey matrix**
- **DNA extraction**
- **PCR-screening**
- **PCR- event specific**
- **Quantification (reference)**

International Workshop

on the consequences of the ECJ judgement on GM pollen in honey for GM crop releases and cultivation in Germany and the EU

Berlin, December 13-14, 2011

Working group report (WG2)

Detection methods for GMP pollen in honey

ANALYTICAL STEP	WHAT WE HAVE	WHAT WE NEED	COMMENT
SAMPLING	DIN 10742:2011 German standard guideline for sampling based on an agreement among stakeholders CODEX sampling standard – not fit for purpose	An international standard taking into account the uncertainty related to inhomogeneity of the sample (especially for big lots/consignments)	need of integration to the current standards including specific situations like shipment of large amounts of honey for a long time span

International Workshop

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Working group report (WG2)

Detection methods for GMP pollen in honey

ANALYTICAL STEP	WHAT WE HAVE	WHAT WE NEED	COMMENTS
Pollen isolation from honey matrix	•Centrifugation (DIN 10760) •Filtration (not standardised)	International standardised efficient procedure providing sufficient amount of pollen suitable for downstream analyses (microscopy or PCR)	Contamination by other plant materials (pollen can't be separated from flours) PCR is not helpful in this case but microscopy is costly .

International Workshop

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Working group report (WG2)

Detection methods for GMP pollen in honey

ANALYTICAL STEP	WHAT WE HAVE	WHAT WE NEED	COMMENTS
DNA extraction	Draft §64 method (German food/feed method collection) Ring trial validation ongoing (to be finalised in January 2012) Extraction commercial kits	An international standardised method providing purified amplifiable DNA (even of less represented species)	Mechanical pre-treatment more efficient than chemical disruption Problem: different resistance to the treatment depending on different pollen structure

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Working group report (WG2)

Detection methods for GMP pollen in honey

ANALYTICAL STEP	WHAT WE HAVE	WHAT WE NEED	COMMENTS
PCR screening	Many validated methods (ISO standards 21569, 21570) .	International harmonised screening approach of testing laboratories.	Not harmonised situation. (different number of elements from one lab to another) but it's a general situation not related specifically to honey matrix
PCR event-specific	EURL-GMFF validated methods		

International Workshop

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Detection methods for GMP pollen in honey

ANALYTICAL STEP	WHAT WE HAVE	WHAT WE NEED	COMMENTS
Quantification (reference)	2 options: Species-specific pollen (microscopy DIN 10740 combined with EURL-GMFF qPCR validated methods) total pollen (*actin* as universal plant reference gene combined with EURL-GMFF qPCR validated methods)	Reference materials Validation and standardisation of the selected option	Microscopy could have a high measurement uncertainty and is costly total pollen counter In principle a PCR solution is preferable

International Workshop

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Detection methods for GMP pollen in honey

Recommendations

1. **Existing national standards need to be contributed to international standardisation level (ISO – CODEX)**
2. **Validated methodologies should be provided on the basis of a clear EC ruling**
3. **More cooperation among testing laboratories (at international level)**

EU funded TRACE project 2005-2009

Tracing the origin of food

TRACE - Molecular Biology Database

Database
- Overview
- Food Products
- Taxonomies
- PCR
- Primers
- Sequences
- Genetic Elements
- Publications
- Organisations
- Samples
- Sample Sites
- *Extractions*

Documentation
Full Documentation

Search
Search Trace

You are here: **Core Froms > Search Sample Extractions**

Overview over all Extraction Records

Show details

Extraction Name	Company	Publication
CTAB		
Dneasy	Qiagen	
E.Z.N.A	Peqlab	
Extraction of DNA from Honey samples	Qiagen	SOP: Extraction of DNA from Honey samples
GeneSpin		
InviSorb	Invitek	
Lyse	Qbiogene	
NucleoSpinFood	Macherey+Nagel	
NucleoSpinTissue	Macherey+Nagel	
Preparation and DNA-Extraction from Honey and Pollen	Biolytix	Preparation & DNA-Extraction from Honey & Pollen
Triton-X100		

http://www.trace.eu.org/mbdb

Interlaboratory validation of a method for the extraction of DNA from pollen in honey

J. Verbr. Lebensm.
DOI 10.1007/s00003-012-0774-z

Journal für Verbraucherschutz und Lebensmittelsicherheit
Journal of Consumer Protection and Food Safety

ANNOUNCEMENTS AND REPORTS

In-house and interlaboratory validation of a method for the extraction of DNA from pollen in honey

Hans-Ulrich Waiblinger · Marc Ohmenhaeuser · Stefanie Meissner · Miriam Schillinger · Klaus Pietsch · Ottmar Goerlich · Joachim Mankertz · Kathrin Lieske · Hermann Broll

Received: 23 April 2012

Abstract In this work, the in-house and interlaboratory validation of a DNA extraction method from pollen in an unifloral rape honey as well as several multifloral honeys is described. The amplifiability of plant and rape DNA amplifiable by real-time PCR was used as a parameter for the evaluation of the method. The practical (i.e., relative) limit of detection was used as a tool for assessing the suitability of the extraction method for further GMO analysis. In a collaborative study with 14 participating labs the results of the in-house validation could be confirmed. The amount of amplifiable plant and rape DNA varied depending on the type of honey. For rape honey, a mean practical LOD of 0.12 % was obtained.

Keywords Honey · DNA extraction · Pollen · Validation · Practical LOD · genetically modified organism (GMO)

Zusammenfassung Die Einzellabor- sowie Ringversuchsvalidierung einer DNA-Extraktionsmethode für Pollen in Honigen wird vorgestellt. Als wesentlicher Parameter für die Auswertung wird die Amplifizierbarkeit von pflanzlicher DNA sowie von Raps-DNA herangezogen, die mittels real-time PCR vervielfältigt werden kann. Die praktische (d. h. relative) Nachweisgrenze wird als Kriterium zur Bewertung der Eignung für eine nachfolgende GVO-Analytik verwendet. In einem Ringversuch mit 14 teilnehmenden Labors wurden die Ergebnisse der In-house-Validie-

Interlaboratory validation of a method for the extraction of DNA from pollen in honey

Table 1 Analysis of genetically modifications in honey, modules and availability of standardized methods

Module	Standardised method available?	Remarks
Sampling	Yes (national level)	DIN 10742 (german standard)
DNA extraction	No	Published method (Waiblinger et al. 1999, 2005) guideline (BVL 2011)
Screening	Yes	E.g. § 64 standards (OC)
Identification of events	Yes	ENGL validated methods (EURL 2011)
Quantification of events	Yes	Only available for quantification of DNA ratio (transgene/species), not for pollen ratios

Interlaboratory validation of a method for the extraction of DNA from pollen in honey

Composition of the honeys :

H1 Honeydew honey with multifloral honey
H2 Wild flower honey (‘‘flowers of the mountains’’)
H3 Wild flower honey
H4 Rape Honey
H5 Acacia-with multifloral honey

Table 2 Participants in the interlaboratory study

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, NRL GVO, Berlin (D)
Bundesinstitut für Risikobewertung, Berlin (D)
Chemisches Landes- und Staatliches Veterinäruntersuchungsamt Münster (D)
Congen Biotechnologie GmbH, Berlin (D)
Eurofins GeneScan GmbH, Freiburg (D)
Genetic ID (Europe) AG, Augsburg (D)
Impetus GmbH & Co. Bioscience KG, Bremerhaven (D)
Kantonales Laboratorium Basel-Stadt, Basel (CH)
Kantonales Laboratorium Zürich, Zürich (CH)
Institut für Hygiene und Umwelt, Hamburg (D)
Landeslabor Berlin-Brandenburg, Berlin (D)
Landesbetrieb Hessisches Landeslabor, Standort Kassel (D)
Landeslabor Schleswig–Holstein, Neumünster (D)
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Lebensmittelinstitut Braunschweig (D)

Interlaboratory validation of a method for the extraction of DNA from pollen in honey

Extraction of DNA from pollen in honey samples

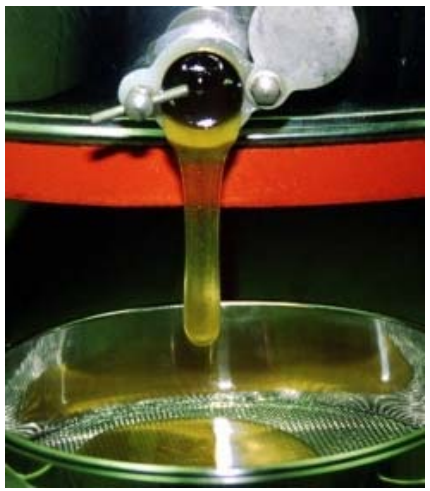
50 g of homogenized honey sample
➔4 equal subsamples + 45 ml TE buffer
➔Centrifugation for 15 min
➔Pellets in 5 ml TE buffer
➔Subsamples were combined
➔Additional centrifugation
➔+ 0.5 ml TE buffer
➔App. 100 mg Glass beeds
➔Incubation o/n
➔Shaking in oscillating mill (mechanical disruption)

➔CTAB extraction

➔Further purification QIAQuick PCR purification kit

Real-time PCR

The applicability of extracted DNA was tested by real-time amplification of
(a)rape resp. Brassica-specific DNA sequences from the *cruciferin A* (cruA) gene (EURL-GMFF 2005)
(b)(b) plant specific sequence from the single copy gene *actin* (Laube et al. 2010)



Interlaboratory validation of a method for the extraction of DNA from pollen in honey

Table 7 Interlaboratory study: real-time PCR systems, master mixes and results of the standard curves

Lab No.	Real-time PCR-system	Mastermix[a]	*actin* PCR		*cruA* PCR	
			Slope	R^2	Slope	R^2
1	ABI 7500	1	−3.20	0.988	−3.58	0.991
2	Rotor Gene 6000	2	**−4.06**	0.985	**−4.49**	0.991
3	ABI 7500	3	−3.46	1.000	−3.52	0.997
4	ABI 7900HT	1	−3.18	0.992	−3.40	0.981
5	ABI 7500 Fast	1	−3.11	0.993	**−3.88**	0.993
6	Stratagene MX3005P	4	**−2.90**	0.989	**−3.62**	**0.975**
7	Rotor Gene Q	5	−3.40	0.998	**−3.88**	0.996
8	ABI 7500	1	−3.15	0.995	−3.53	0.986
9	ABI 7900HT	6	−3.13	**0.978**	−3.27	0.991
10	Stratagene MX3005P	6	−3.10	0.991	−3.30	0.989
11	ABI 7500	7	−3.48	0.999	**−3.98**	0.996
12	ABI 7500	1	**−2.72**	0.994	−3.18	0.999
13	Roche LC 480	1	**−4.00**	0.997	**−4.00**	0.983
14	ABI 7500	1	−3.44	0.999	−3.37	0.986

In bold: slope of rape DNA dilution series greater than −3.1 or less than −3.6 resp. $R^2 < 0.98$

[a] Master mixes: 1, Universal PCR Master Mix (Applied Biosystems); 2, Quantitect Multiplex no rox (QIAGEN); 3, Reagents not commercially available; 4, Sure Master (Congen); 5, LC480 Probes Master (Roche); 6, Eurogentec qPCR Mastermix; 7, Quantitect (QIAGEN)

Interlaboratory validation of a method for the extraction of DNA from pollen in honey

Table 9 Results of the ring-trial for extraction of DNA from 5 honeys (H1–H5)

	H1	H2	H3	H4	H5
Results *actin* PCR (plant)					
Number of positive reactions/reactions total	84/84	84/84	84/84	84/84	84/84
Ct range	26.1–31.4	27.1–30.3	28.1–30.4	23.4–27.7	28.7–32.4
Copy number mean (per PCR)	2,198	1,100	561	11,718	354
Copy number median (per PCR)	729	839	346	10,658	304
Cv copy number (%)	145	68	109	46	79
Results *cruA* PCR (rape)					
Number of positive reactions/reactions total	82/84	84/84	83/84	84/84	84/84
Ct range	33.9–40.2	34.1–41.0	33.9–39.7	24.9–29.6	30.9–38.5
Copy number mean (per PCR)	22	<20	<20	8,013	173
Copy number median (per PCR)	<20	<20	<20	5,604	120
Cv copy number (%)	93	112	107	75	101
Practical LOD (%)	**>50**	**>50**	**>50**	**0.12**	**6**

Interlaboratory validation of a method for the extraction of DNA from pollen in honey

Table 10 Ring-trial, results for honey 4 (rape honey), cruciferin A PCR

Lab no.	Ct	Number of positive reactions/total reactions	*cruA* copies	s_r copies	$s_{r,\ rel}$, % copies	*cruA* copies, mean	s_R copies	$s_{R,\ rel}$, % copies	prLOD (%)	prLOD (%) mean
1	28.9	84/84	5,450	356	6.5	8,013	5,979	75	0.18	0.12
2	27.1		5,611	1,266	22.6				0.18	
3	24.9		5,596	165	3.0				0.18	
4	29.5		3,393	991	29.2				0.31	
5	27.4		8,599	384	4.5				0.12	
6	27.2		19,970	8,113	40.6				0.06	
7	26.8		5,654	1,119	19.8				0.18	
8	26.3		9,052	1,265	14.0				0.11	
9	28.4		5,482	839	15.3				0.19	
10	27.2		4,243	1,493	35.2				0.26	
11	26.6		6,319	1,047	16.6				0.16	
12	29.6		5,101	808	15.8				0.20	
13	26.9		22,432	631	2.8				0.04	
14	27.7		5,279	1,018	19.3				0.19	

s_r, repeatability standard deviation, absolute (in copies per PCR); $s_{r,\ rel}$, repeatability standard deviation, relative (in %); s_R, reproducibility standard deviation, absolute (in copies per PCR); $s_{R,\ rel}$, reproducibility standard deviation, relative (in %); prLOD, practical LOD (in %); assumption, LOD of transgene specific PCR is 10 copies

Matrix approach in conjunction with pre-spotted 96-well plates

	Primer / Probe	*Sequence*
act	act-f	CAA gCA gCA TgA AgA TCA Agg T
	act-r	CAC ATC TgT Tgg AAA gTg CTg Ag
	act-probe	CCT CCA ATC CAg ACA CTg TAC TTY CTC TC -TMR
Rice	KVM159	TGGTGAGCGTTTTGCAGTCT
	KVM160	CTGATCCACTAGCAGGAGGTCC
	TM013	TGTTGTGCTGCCAATGTGGCCTG
Maize	adh1 primer 1	CCAGCCTCATGGCCAAAG
	adh1 primer 2	CCTTCTTGGCGGCTTATCTG
	adh1 probe	CTTAGGGGCAGACTCCCGTGTTCCCT
Rape seed	Pep-F	CAGTTCTTGGAGCCGCTTGAG
	Pep-R	TGACGGATGTCGAGCTTCACA
	Pep-sonde	6FAM-ACAGACCTACAGCCGATGGAAGCCTGCXTp
Soya	GM1-F	CCAGCTTCGCCGCTTCCTTC
	GM1-R	GAAGGCAAGCCCATCTGCAAGCC
	GM1-Sonde	FAM---CTTCACCTTCTATGCCCCTGACAC--TMR
p35s	35S-F	GCCTCTGCCGACAGTGGT
	35S-R	AAGACGTGGTTGGAACGTCTTC
	35S-TMP	6FAM-CAAAGATGGACCCCCACCCACGTXTp
t-NOS	180-F (T-nos-F)	CATgTAATgCATgACgTTATTTATg
	180-R (T-nos-R)	TTg TTT TCT ATC gCg TAT TAA ATg T
	Tm-180 (T-nos-P)	ATgggTTTTTATgATTAgAgTCCCgCAA
bar	RapB-F1	ACA AGC ACG GTC AAC TTC C
	RapB-R1	GAG GTC GTC CGT CCA CTC
	RapB-S1	6-FAM-TAC CGA GCC GCA GGA ACC-TAMRA
ctp2-epsps	Ctp2-cp4epsps-F	GGGATGACGTTAATTGGCTCTG
	Ctp2-cp4epsps-R	GGCTGCTTGCACCGTGAAG
	Ctp2-cp4epsps-P	CACGCCGTGGAAACAGAAGACATGACC
p35s-pat	P35S-pat-f	AAgTTCATTTCATTTggAgAggACA
	P35S-pat-r	CggCCATATCAgCTgCTgTAg
	P35S-pat-Probe	CCGGAGAGGAGACCAGTTGAGATTAGGC

Waiblinger, H.-U., Grohmann, L., Mankertz, J., Engelbert, D. and K. Pietsch (2010). A practical approach to screen for authorised and unauthorised genetically modified plants. Anal Bioanal Chem (2010) 396:2065–2072.

Plate design of pre-spotted 96-well plates for screening

	1	2	3	4	5	6	7	8	9	10	11	12	
A	LL62	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10	Reis 200Ge/µl	Rice
B	Bt176	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10	Mais 200Ge/µl	Maize
C	Rapeseed (aus Blättern)	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10		Rapeseed
D	RRS 5%	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10		Soya
E	RRS 5%	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10	Bt11 200Ge/µl	p35s / t-NOS
F	Bt176	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10	Bt176 200 Ge/µl	bar
G	NK603	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10	NK603 200 Ge/µl	ctp2-epsps
H	T25	NK	C11098BL -a 1:10	C11098BL -a 1:10	C11098BL -b 1:10	C11098BL -b 1:10	C11099BL -a 1:10	C11099BL -a 1:10	C11099BL -b 1:10	C11099BL -b 1:10	AK 1:10	LL62 200 Ge/µl	p35s-pat
	1	2	3	4	5	6	7	8	9	10	11	12	

Zagon, J., Kurth, S., Ehlers, A., Linke, B., Lampen, A. and Hermann Broll. Preservation of primer and probes on “ready-to-use” 96-well microtiter plates: A step forward towards enhancing throughput and harmonization of real-time PCR applications in food and feed control (2012). Food control: **25** (2): 709–716

Results for honey samples with pre-spotted 96-well plates

Sample no	Honey samples	Origin	Company	p35s / t-NOS	bar	ctp2-epsps	35s-pat	Rice	Maize	Rapeseed	Soya
C11093BL	Rapeseed honey	Canada	Biophar *Vertrieb:* Dr. med. Hans Plümer Nachf. D-38110 Braunschweig	X	X	X	-	-	-	X	-
C11094BL	Rapeseed honey	EU and non-EU countries	Christians Grod Scandic Food A/S DK-7100 Vejle	-	-	-	-	-	-	X	-
C11095BL	No information	Non-EU countries	Florimel 8630 Veume (Belgien) 17153 Stavenhagen	-	-	-	-	-	-	X	-
C11096BL	from meadow	EU and non-EU countries	BioGourmet aus kontrolliert biologischer Imkerei 87730 Bad Grönenbach	-	-	-	-	-	-	X	-
C11097BL	Rapeseed honey	EU and non-EU countries	Dr. med. Hans Plümer Nachf. GmbH & Co. KG Am Salgenholz 2 38110 Braunschweig-Wnden	X	X	X	-	-	-	X	-
C11098BL	Gelee Royale	No information	Dr. med. Hans Plümer Nachf. GmbH & Co. KG Am Salgenholz 2 38110 Braunschweig-Wnden	-	-	-	-	-	-	-	-
C11099BL	Rapeseed honey	EU countries	Langnese Honig 22933 Bargtheheide	-	-	-	-	-	-	X	-

X	positive result
-	negative result

Results for honey samples with pre-spotted 96-well plates

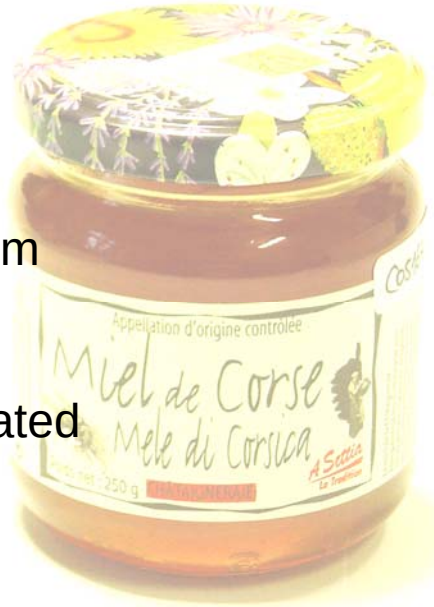
	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed rape Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	Bt11 Maize	NK603 Maize	GA21 Maize method 1	MON863 Maize	1507 Maize
B	T25 Maize	59122 Maize	H7-1 Sugarbeet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 Rice	T45 Oilseed rape	EH92-527-1 Potato	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 Oilseed rape
C	LLCotton25 Cotton	MON 531 Cotton	A2704-12 Soybean	MIR604 Maize	Rf1 Oilseed rape	Rf2 Oilseed rape	Ms1 Oilseed rape	Topas 19/2 Oilseed rape	MON1445 Cotton	Bt176 Maize	MON15985 Cotton	40-3-2 Soybean
D	GA21 Maize method 2	MON88017 Maize	LY038 Maize	3272 Maize	MON89788 Soybean	MON89034 Maize	DP-356043 Soybean	MON88913 Cotton	Rice GM events P35S::bar	LLRice601 Rice	Bt63 Rice	Bt10 Maize
E	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed rape Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	Bt11 Maize	NK603 Maize	GA21 Maize method 1	MON863 Maize	1507 Maize
F	T25 Maize	59122 Maize	H7-1 Sugarbeet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 Rice	T45 Oilseed rape	EH92-527-1 Potato	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 Oilseed rape
G	LLCotton25 Cotton	MON 531 Cotton	A2704-12 Soybean	MIR604 Maize	Rf1 Oilseed rape	Rf2 Oilseed rape	Ms1 Oilseed rape	Topas 19/2 Oilseed rape	MON1445 Cotton	Bt176 Maize	MON15985 Cotton	40-3-2 Soybean
H	GA21 Maize method 2	MON88017 Maize	LY038 Maize	3272 Maize	MON89788 Soybean	MON89034 Maize	DP-356043 Soybean	MON88913 Cotton	Rice GM events P35S::bar	LLRice601 Rice	Bt63 Rice	Bt10 Maize

Querci, M., Foti, N., Bogni, A., Kluga, L., Broll, H. and Guy Van den Eede. Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection (2009). Food Analytical Methods: **2:**325–336.

Using EURL-GMFF pre-spotted plates it was possible to determine the event responsible for the positive signals in the screening plate

RT73

Recommendations



Honey intended for export into the EU, in which the absence of gm pollen can not be guaranteed needs to be tested

DNA extraction should be done using the collaborative trial validated procedure in order to be in-line with ISO 17025

Testing using a screening approach might be advisable

In case samples are positive for EU authorised GMOs the honey will need a label if the presence is above the threshold

Quantification is technically only possible if relative quantification of transgenic DNA related to species DNA is considered

In case the GMO identified is not authorised in the EU the honey is also not authorised on European Union market and consequently can not be exported to the EU

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Thank you for your attention

Hermann Broll

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