SLOVENSKI STANDARD
SIST EN ISO 21569:2005/A1:2013

01-junij-2013

Živila - Analitske metode za odkrivanje gensko spremenjenih organizmov in njihovih produktov - Kvalitativne metode na osnovi nukleinske kisline - Dopolnilo A1 (ISO 21569:2005/Amd 1:2013)

Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods - Amendment 1 (ISO 21569:2005/Amd 1:2013)

Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Qualitative auf Nukleinsäuren basierende Verfahren - Änderung 1 (ISO 21569:2005/Amd 1:2013)

Produits alimentaires - Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés - Méthodes qualitatives basées sur l'utilisation des acides nucléiques - Amendement 1 (ISO 21569:2005/Amd 1:2013)

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ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

SIST EN ISO 21569:2005/A1:2013 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

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EN ISO 21569:2005/A1

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English Version

Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods - Amendment 1 (ISO 21569:2005/Amd 1:2013)

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Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Qualitative auf Nukleinsäuren basierende Verfahren - Änderung 1 (ISO 21569:2005/Amd 1:2013)

This amendment A1 modifies the European Standard EN ISO 21569:2005; it was approved by CEN on 14 March 2013.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN ISO 21569:2005/A1:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 “Food analysis - Horizontal methods” the secretariat of which is held by DIN.

This Amendment to the European Standard EN ISO 21569:2005 shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2013, and conflicting national standards shall be withdrawn at the latest by October 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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Endorsement notice

The text of ISO 21569:2005/Amd 1:2013 has been approved by CEN as EN ISO 21569:2005/A1:2013 without any modification.

INTERNATIONAL STANDARD

ISO 21569

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2005-06-15

AMENDMENT 1
2013-04-01

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods

AMENDMENT 1

Produits alimentaires — Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés — Méthodes qualitatives basées sur l'utilisation des acides nucléiques

AMENDEMENT 1

Reference number
ISO 21569:2005/Amd.1:2013(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

Amendment 1 to ISO 21569:2005was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods

AMENDMENT 1

No attempt has been made in this amendment to update the footnote numbering to fit in with the scheme adopted in ISO 21569:2005. The footnote numbers given are for use refer solely within this amendment.

Page v, Introduction, paragraph 1

Delete "— *Sampling* (ISO 21568)".

Page 2, Clause 2, ISO 24276

Delete the footnote and update the entry to read:

ISO 24276:2006, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

Page 2, 4.1, paragraph 2

Delete the existing text and insert the following.

A qualitative result shall clearly demonstrate the presence or absence of the genetic element under study, relative to appropriate controls.

NOTE Detection limits and size of the test portion are critical aspects of a method.

Page 2, 7.3.3.3.3, paragraph 2

Delete "a representative" and insert "an appropriate" so that the text reads as follows.

Primers designed to detect taxon-specific target sequences should be shown to detect these sequences reliably in an appropriate number of different members of the taxon.

Page 6, 8.1 a) and b)

In both cases, delete "ISO 24276:—", and insert "ISO 24276:2006.

Page 7, 9.4

Delete the existing text and insert the following.

Results within the same test portion shall be consistent. In case of +/− results for the two replicates, repeat the two PCR for the respective test portion. If the two novel replicates are tested +/− or −/−, the test portion is considered as negative.

Results from all test portions shall be consistent. When at least one test portion gives a positive result and at least one gives a negative result, the analysis shall be repeated.

If at least one repetition of the procedure, beginning with the nucleic acid extraction, gives ambiguous results such as a positive and a negative result, the report should state that the sample is negative at the limit of detection (LOD).

Page 7, Clause 10, list item 2

Delete the existing text and insert the following.

— the specificity of the analytical method (event specific, construct specific, or screening method);

Page 23, Annex A

Insert A.5 and A.6 after the existing text.

A.5 Target taxon-specific method for the detection of DNAs derived from rice

A.5.1 Purpose, relevance and scientific basis

The GMO Detection Laboratory of Shanghai Jiao Tong University (GMDL-SJTU) organized a collaborative study for validation of the applicability of a target taxon-specific method using the rice sucrose phosphate synthase (*SPS*) gene as an endogenous gene for qualitative analysis of genetically modified (GM) or non-GM rice. This study involved 12 laboratories from Spain, Korea, Lithuania, Slovenia, Japan, Italy, and China.

The operational procedure of the collaborative study comprised the following modules:

— qualitative PCR for validation of the heterogeneity of the *SPS* gene among rice cultivars for different geographic and phylogenetic origins;

— qualitative PCR for validation of the species specificity of *SPS* gene for rice;

— qualitative PCR for evaluation of the LOD of the established *SPS* qualitative PCR assay.

The collaborative study was carried out in accordance with Reference [44].

The results of the collaborative study as well as the related protocol are given in A.5.3.

A.5.2 Principle

The method has been optimized for rice seeds and other processed products such as seed powder. Applicability of the *SPS* gene was evaluated in this collaborative study using DNA samples extracted from rice seeds and other plant materials.

The collaborative study organizer provided method-specific reagents (primers, probes, reaction master mix), and the test DNA samples extracted from rice materials to collaborative study participants.

A.5.3 Validation status and performance criteria

A.5.3.1 Robustness of the method

Robustness has been tested on the *SPS* gene qualitative PCR system for three different annealing temperatures (i.e. 56 °C, 58 °C, and 60 °C), on three different DNA samples containing known amounts of rice DNA (10 ng, 1 ng, 0,1 ng rice genome DNA samples) and with three repetitions per sample. The qualitative PCR systems demonstrated the expected robustness and performed well at all three annealing temperatures and three concentrations of the rice DNA samples.

The *SPS* gene qualitative PCR system was also tested on different thermal cyclers (PTC-100,[1)] MJ Research and instruments from Bio-Rad and Applied Biosystems), on three different reaction volumes (25 µl, 30 µl, and 50 µl) and three repetitions per volume. The qualitative PCR systems had the expected robustness and performed well on different thermal cyclers and with different reaction volumes.

A.5.3.2 Intralaboratory trial

The rice *SPS* gene has been described as being suitable for use as an endogenous reference gene in rice identification and quantification (Reference [44]). The detailed technical information was modified from Reference [44].

For sample preparation in the collaboration study, all the DNA samples were extracted by the GMDL-SJTU using the CTAB method adopted from ISO 21571:2005, A.3. Spectrophotometric quantification of DNA extracted was performed using a method adopted from ISO 21571:2005, B.1. After the DNA quantification, a qualitative PCR using an 18S PCR system (Reference [45]) was carried out to provide data about possible PCR inhibition.

The *SPS* gene PCR system was tested using rice genomic DNA by three researchers at the GMDL-SJTU. The results were satisfactory; in particular, for qualitative PCR, the results show that the *SPS* gene is specific for rice, and the LOD is about 0,1 %.

A.5.3.3 Collaborative trial

For the collaborative study, each participant received 12 rice DNA samples for heterogeneity testing; 10 DNA samples from plants other than rice plus one DNA sample from rice for species specificity testing; and 10 serially diluted rice samples for LOD evaluation. A negative and a positive control were also included.

The heterogeneity of the *SPS* gene among rice cultivars was evaluated using 12 rice cultivars from different geographic and phylogenetic origins in China, such as Najing14, Taibei309, Shengnong265, Jinyinbao, Minghui78, Huke3, Guangluai4, Zhe733, Hejiang19, Baizhehu, Xiangwanxian9 and Nipponbare. The results returned from 12 laboratories showed that out of a total of 144 (12 × 12) rice DNA samples, 143 positive results were obtained using the *SPS* gene PCR system. This means that the false-negative rate of the *SPS* gene PCR system for rice is 0,69 % (1/144) (see Table A.14). These data suggest that there is low heterogeneity of the *SPS* gene in the target region.

The species specificity of the *SPS* gene was validated using a rice genome DNA sample (Guangluai4) and 10 other plant DNAs that were evolutionarily related to rice, common crops or model plants, such as the fruit materials of bamboo (*Phyllostachys* spp.), green bristlegrass [*Setaria viridis* (L.) Beauv.], barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), foxtail millet (*Setaria italica*), rapeseed (*Brassica napus*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), soya bean (*Glycine max*) and thale cress (*Arabidopsis thaliana*). The results returned from 12 laboratories showed that out of a total of 120 (10 × 12), non-rice plant DNA samples, 118 negative results were obtained using the *SPS* gene PCR system. This means that the false-positive rate of the *SPS* gene PCR system for other 10 plant materials was 1,67 % (2/120) (see Table A.14). These data suggest that the *SPS* gene is species specific for detection of rice.

1) Example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table A.14 — The results of heterogeneity and of specificity testing of the qualitative PCR

Parameter (collaborative study of 2007)	Value
No. laboratories	12
No. laboratories submitting results	12
No. samples per laboratory	22
No. accepted results	264
No. samples containing rice	144
No. samples not containing rice	120
False-positive results	2 (1,67 %)
False-negative results	1 (0,69 %)

The LOD of the *SPS* gene PCR system was validated using mixed powder containing maize and various quantities of rice seed by means of qualitative PCR: all 12 laboratories detected the SPS gene in the DNA sample extracted from mixed powder containing 0,1 % mass fraction or higher of rice, and two in 12 laboratories detected it from mixed powder containing 0,01 % mass fraction of rice. These data suggest that the LOD of the *SPS* gene PCR system is as low as 0,1 % mass fraction (see Table A.15).

Table A.15 — The results of the LOD test of the qualitative PCR

Parameter (collaborative study of 2007)	Rice to maize mass fraction, m_{rice}/m_{maize}				
	10 %	1 %	0,1 %	0,05 %	0,01 %
No. laboratories	12	12	12	12	12
No. laboratories submitting results	12	12	12	12	12
No. samples per laboratory	2	2	2	2	2
No. laboratories accepted results	12	12	12	12	12
Positive results	12 (100 %)	12 (100 %)	12 (100 %)	4 (33,33 %)	2 (16,67 %)

A.5.3.4 Molecular selectivity

A.5.3.4.1 General

For qualitative validation of the *SPS* gene as a specific rice gene, a 279 bp fragment of the conserved region of the *SPS* gene was selected and amplified using specific primers.

A.5.3.4.2 Experimental

DNA samples extracted from 11 different plant materials (including rice) were analysed by the *SPS* gene PCR system as described (Reference [44]). Among the 11 samples, only rice DNA gave positive results. The other 10 samples (see A.5.3.3) gave negative results.

The DNA samples extracted from 12 different rice cultivars were analysed by the *SPS* gene PCR system reported in Reference [44]. All 12 samples gave positive results.

A.5.3.4.3 Theoretical

The theoretical specificity of the *SPS* gene primer was assessed through a homology search using the BLASTN 2.0MP-WashU program (Reference [82], search date: 2010-01-09). The 279 bp sequence used as query is part of the NCBI accession number U33175 (nucleotides 1055–1333). The results of the basic local alignment search tool (BLAST) confirmed the complete identity of the query sequence with rice *SPS* gene sequence, and no homology with other genes and species.

A.5.4 Principle and summary

This methodology is a PCR procedure for the applicability of the *SPS* gene for use as a rice endogenous gene in qualitative detection of GM or non-GM rice. Heterogeneity, species specificity of the *SPS* gene and LOD were evaluated as part of the validation of this method. The 279 bp PCR product was visualized by agarose gel electrophoresis.

A.5.5 Terms and definitions

For the purposes of this document, the terms and definitions of ISO 5725-1[40] and ISO 24276 apply.

A.5.6 Sample type and amounts

In the following, the data from the collaborative study are given as examples for sample types and sample amounts adequate for this method.

DNA samples extracted from the seeds of 12 rice cultivars, 10 other plant materials (see A.5.3.3) and the mixed powder containing different mass fractions of rice in maize seed power, were used in this collaborative study.

The participants received the following samples.

— 12 DNA samples from 12 different rice cultivars that are widely planted in different region of China (i.e. Najing14, Taibei309, Shengnong265, Jinyinbao, Minghui78, Huke3, Guangluai4, Zhe733, Hejiang19, Baizhehu, Xiangwanxian9, and Nipponbare), 20 ng/µl, 50 µl each. These DNA samples were used to validate the heterogeneity of the *SPS* gene among rice cultivars.

— 11 DNA samples from rice (Guangluai4) and 10 other plant materials which are related to rice (i.e. bamboo, green bristlegrass, barley, wheat, foxtail millet) or common GM crops (i.e. rapeseed, tomato, potato and soya bean) or model plants (i.e. thale cress), 20 ng/µl, 50 µl each. These DNA samples were used to validate the species specific of the *SPS* gene in rice.

— 10 DNA samples from mixed powders of maize with different mass fractions of rice, 20 ng/µl, 50 µl each. These DNA samples were double blind replicates of the series of five rice concentrations used for testing the LOD of the *SPS* gene PCR system.

— Negative DNA target control (labelled N): salmon sperm DNA (20 ng/µl).

— Positive DNA target control (labelled P): rice (Guangluai4) genomic DNA (20 ng/µl). All the DNA samples were purified using the CTAB method by the GMDL-SJTU. The negative and positive DNA target controls were used for each PCR plate.

— Reaction reagents, primers for the *SPS* gene PCR system as follows:

  — primer pair for conventional PCR: SPS-F/SPS-R;

  — DNA dilution solution [0,1× tris–EDTA (TE), 1,2 ml].

A.5.7 Limit of detection and range of use

DNA was extracted from five mixed powder samples containing different amounts of rice. These samples were analysed by the SPS PCR system as described (Reference [44]). Positive results were obtained with samples containing mass fractions of 10 %, 1 %, and 0,1 % rice. The other two samples (containing mass fractions of 0,05 % and 0,01 %) gave negative results.

According to the developed method, the relative LOD of the qualitative PCR method is about 0,1 % mass fraction. The *SPS* gene PCR system can be used for specific detection and identification of rice materials in other plant materials.