

**Final report of workpackage 5:
“Validation of techniques in ring trials”**

Project title:

**“Reliable, standardised, specific, quantitative detection of
genetically modified food”**

Acronym: Qpcrgmofood

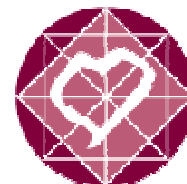


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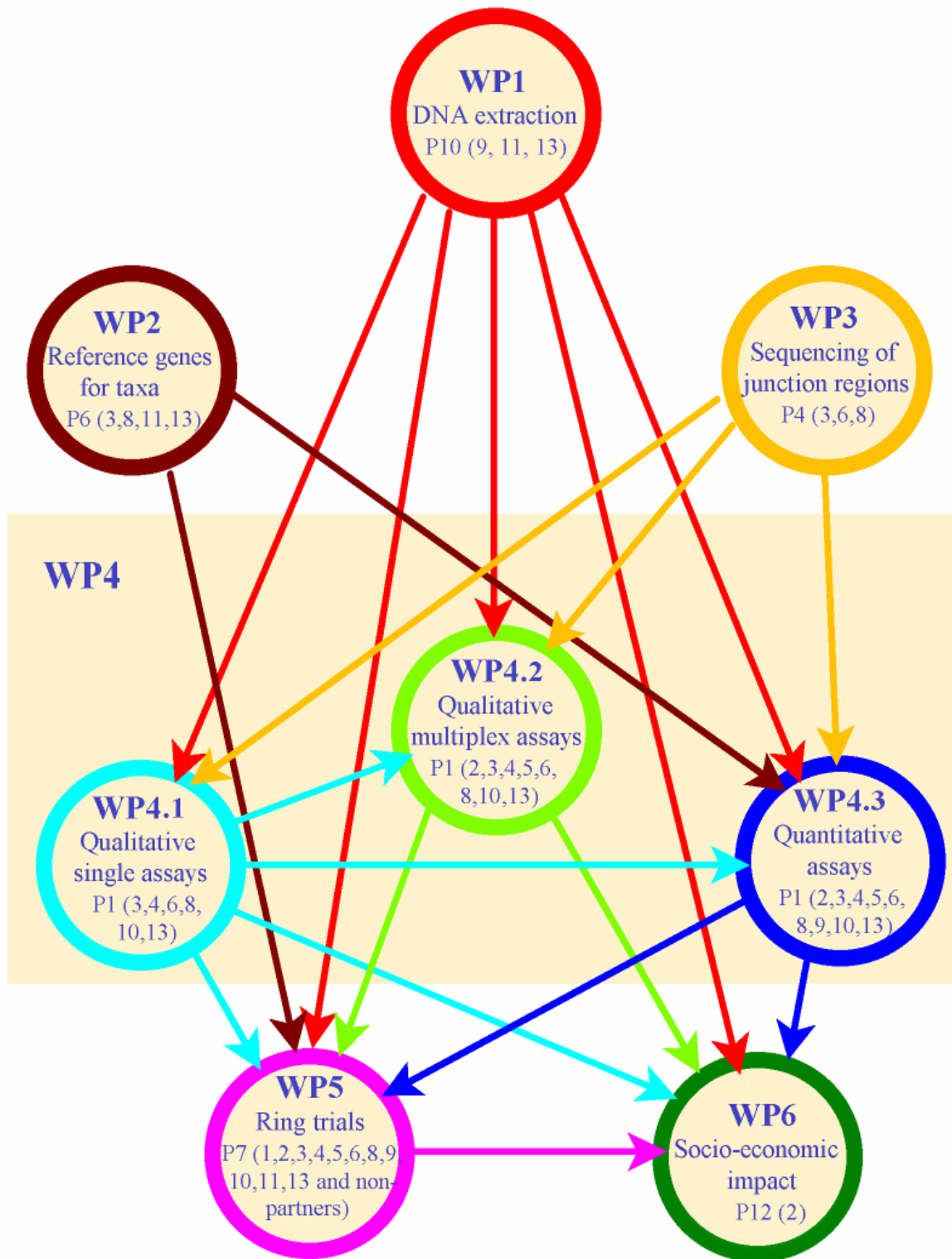
Project website: www.vetinst.no/Qpcrgmofood/Qpcrgmofood.htm

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DELIVERABLE NUMBER 7

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Outline of project structure, showing links between workpackages and partners

Summary

In general, a large number of methods are currently being developed and published for GMO detection, identification and quantification. Before they are introduced into use they must be validated so that we can have a confidence that the methods consistently meet the requirements for their intended applications. The aim of the Work Package 5 “Validation of methods in ring-trials” has been to validate in ring-trials the most suitable techniques developed within the project.

Besides the method validation as such, the approaches on how to organise the validation procedure have been developed further. In particular, the project has very much contributed to the development, testing and implementation of the modular validation approach, in which the extraction methods, reference gene systems and GMO-specific systems can be validated separately.

Due to the difficulties encountered in the material availability, sequencing and method development, the WP activities started with a considerable delay. The majority of the ring-trials were carried out during the period 1.8.2002 – 31.7.2003. The validated protocols were methods for DNA extraction, reference gene systems and event-specific quantitative real-time PCR methods for different maize, soy and rapeseed matrices. Altogether 8 protocols underwent a single-laboratory pre-validation study, 20 protocols a multi-laboratory validation study with 2 – 5 participating laboratories and six protocols a full collaborative ring-trial. Thus, 34 different studies were organised within the WP validation. In addition, two full collaborative trials started just after the project end, and are currently coordinated by the JRC (Bt11 and T25 event-specific real-time PCR methods). The majority of the protocols tested performed well, in some cases further optimisation of the protocols was recommended and in some cases the validation studies were hampered due to the poor quality of the reagents or of the DNA.

The experience gained in this scale about validation studies is extraordinary. The effort both from the WP leader, who has coordinated and organised all studies, prepared and tested all the materials and carried out all the data analysis as well as from the participating laboratories has been very high and intensive. It is clear that the contributions are clearly over anything which has been directly financed by the project. The efforts and the motivation have arisen from the general enthusiasm and good spirit within the project and among its participants but also from the very large interest shown in the project and in the availability of validated methods by the European GMO testing community. In addition, the experience gained within this workpackage has been beneficial for the drafting of the European standards within the CEN, for the European Network of GMO Laboratories, which is drafting a document defining minimum performance criteria for validated methods to be accepted as fit for regulatory compliance (see <http://engl.jrc.it/>) and for the JRC as it is preparing to become the European Community Reference Laboratory for GMO testing with the main task of method validation (established in the Regulation EC 1829/2003).

Overview of partners, workplan and deliverables of WP5

The validation activity has been very much a joint effort within the project, and therefore practically all the project participants (excluding P12 who was not involved in analytical work) have been involved in it. In full ring-trials, also laboratories outside of the project consortium have participated.

The following partners participated in the work performed in WP5:

- P7 European Commission, DG Joint Research Centre, Ispra, Italy (*WP task leader*)
- P1 National Veterinary Institute, Oslo, Norway (*project coordinator*)
- P2 Norwegian Food Research Institute, Aas, Norway
- P3 Institut National de la Recherche Agronomique, Versailles, France
- P4 Department of Plant Breeding, Center of Agricultural Research, Melle, Belgium
- P5 LGC Ltd., Teddington, U.K.
- P6 GeneScan GmbH, Freiburg, Germany
- P8 Tepral, Strasbourg, France
- P9 Unilever, Vlaardingen, the Netherlands
- P10 DGCCRF-LIR67, Strasbourg, France
- P11 Bio-Geves, Surgeres, France
- P13 Consejo Superior de Investigaciones Cientificas, Barcelona, Spain

The workplan of WP5 (as in the project proposal and contract):

Once suitable and well performing sequences/techniques for identification/detection of single taxa/GMOs are identified and their satisfactory performance confirmed by at least three laboratories, a ring-trial will be prepared. Ring trials will be set up after 12, 24, 30 and 36 months.

Objectives (as in the contract)

Validation of developed techniques in ring-trials including at least 20 independent laboratories. After validation the techniques are submitted to CEN for standardization at the European level.

Deliverable (No. 7):

The Deliverable from the Work Package is a publicly available report on the “Validation of DNA extraction technique and of the use of methods/sequences developed by collaborating laboratories to qualitatively or quantitatively detect GMOs at different levels of specificity”.

Introduction to the activities

In general, a large number of methods are currently being developed and published for GMO detection, identification and quantification. Before they are introduced into use they must be validated so that we can have a confidence that the methods consistently meet the requirements for their intended applications. The aim of the Work Package 5 “Validation of methods in ring-trials” has been to validate in ring-trials the most suitable techniques developed within the project. After the validation, the results will be published and, where appropriate, submitted to CEN for standardization at the European level.

Due to the difficulties encountered in the material availability, sequencing and method development, the WP activities started with a considerable delay. This means that the majority of the ring-trials were carried out during the period 1.8.2002 – 31.7.2003. Therefore, a number of scientific publications describing the results will be published after the end of the project duration. In addition, some of the methods developed in the project are currently under full collaborative trial or will be considered for the validation in the future due to the general interest in method availability.

Development and implementation of the validation scheme

The method validation process includes the definition of the requirements for the method, testing that the method meets these requirements when carried out, for instance, by different laboratories in different countries, and documentation of the method performance and measurement uncertainty. Finally, the validated method protocols will be submitted to the user community and eventually for international standardization bodies.

A large-scale collaborative trial is expensive and usually follows only after the method has shown acceptable performance in an in-house testing and in a pre-validation study. Formal validation of a method is the conclusion of a long process, which includes the following main steps:

- *Method development and method optimization.* Prior to validation activities, the method should be fully optimized so that an inter-laboratory transfer is possible. The protocol should be finalized so that no major changes are needed between the pre-validation and validation.
- *Multi-laboratory testing of the method (pre-validation),* commonly involving 2 – 4 laboratories. During the project it became clear that external laboratories should be involved at this stage in order to test the inter-laboratory transferability of the method in contrast to the in-house validation by the method developer.
- *Full collaborative trial of the method (also called full validation, full ring-trial or formal validation).* Full collaborative trial requires considerable resources and should be conducted only on methods, which have received adequate prior testing.

There are several different definitions of a validation study. A multi-laboratory testing of the method can already be considered as a kind of validation study, provided that the experimental design and organisational procedures applied fulfil certain minimum requirements. Within this project, similar experimental design and organisational arrangements (e.g. blind samples) were applied in the multi-laboratory testing and in full collaborative trials.

The CEN prEN ISO 24276:2002 (E) provides the following definition for a collaborative trial:

“A study in which several laboratories measure a quantity in one or more “identical” portions of homogeneous, stable materials under documented conditions, the results of which are compiled

into a single document. Guidelines for performing collaborative trials are elaborated by ISO 5725 and ISO/AOAC/IUPAC harmonized protocol¹.”

Accordingly, a collaborative study should report data from at least 8 laboratories for quantitative methods in order to be proposed as a CEN standard.

The following draft CEN standards have in particular been taken into account in the planning of the validation activities within this project:

- General requirements and definitions: Draft European standard prEN ISO 24276:2002;
- Nucleic Acid extraction methods: Draft European standard prEN ISO 21571:2002;
- Quantitative nucleic acid based methods: Draft European standard prEN ISO 21570:2002;
- Protein based methods: Draft European standard prEN ISO 21572:2002;
- Qualitative nucleic acid based methods: Draft European standard prEN ISO 21569:2002.

Description of the organisation of the validation studies

In order to face the challenges of the validation work – and due to the experience obtained during the project – P7 (WP coordinator) has further developed the laboratory processes necessary to organise ring-trials on a larger scale, and to prepare and test the materials to guarantee the high quality throughout. The importance of high quality in all contributions was emphasised in some lessons gained during the project. The results of some studies have clearly suffered due to the poor quality of reagents and DNA and due to the lack of very detailed control of a protocol submitted to the trial participants. Furthermore, in the absence of accurate and standardized methods to measure DNA quantity, the preparation of genomic DNA samples requires extreme care.

Primary protocols have been prepared by the method developers. These have been reviewed by the JRC and transformed into harmonized validation protocols. This means that, for example, all details about how to handle the materials, set-up the reactions to minimize errors and contaminations, the details about the sample coding, experimental design and data storage and submission have been inserted. The experimental design has been set to comply with the CEN requirements so that the methods can be submitted for CEN standardization after a successful validation.

The multi-laboratory (pre-)validation studies have been carried out by involving 2 – 5 project participants. The laboratory, which has developed the method acted among these as an internal control, because they can immediately report if, for one reason or another, the method is not performing in the same ways as in in-house testing. The participating laboratories have been selected according to their interests and, furthermore, by trying to balance the workload.

The responsibilities for the provision of sample materials were divided among the trial participants, and the method developer has been responsible for the provision of the reagents. The JRC estimated the amount of materials needed, and all the materials were provided to the

¹ Horwitz, W. (1995). Protocol for the design, conduct and interpretation of method performance studies. *Pure and Applied Chemistry* **67**: 331-343.

JRC. The JRC developed an approach that was used to prepare and test all the materials prior to the launch of the validation studies. Preparation of materials includes, for instance, material checking, sample preparation, aliquoting, dilution, labelling and packing of the materials as well as the shipping of the materials to the ring-trial participants. This is a very crucial and time-demanding working step, and a failure at this stage affects directly the results of the ring-trials. Thus, the preparations must be carried out by experienced personnel and with extreme care. Enhanced quality control rules especially for the genomic DNA and primers and probes have been introduced for the JRC in-house testing and the preparation of materials.

After the material testing and in-house testing of the protocols, the JRC distributed the sample material and corresponding reagents to the participating laboratories. The automatic output modes of the analysis equipment were utilized in the data output as much as possible. The data are provided to the JRC. The JRC took care for the data storage and analysis. In practice, all the experimental results have been available with a two – six weeks' delay, depending of the trial. The analysis of the results has been delayed accordingly. The JRC is responsible for the data analysis and reporting.

The importance of delivering the different contributions and experimental results in time was clearly emphasised due to the tight timetable of the validation WP.

Introduction to the modular approach of validation

The project has very much contributed to the development, testing and implementation of the modular validation approach (Holst-Jensen and Berdal, 2003), in which the extraction methods, reference gene systems and GMO-specific systems can be validated separately. Concurrently, the approach has been adopted by the CEN/TC 275/WG 11 and is suggested to form the frame for the standardization of GMO testing methods within Europe and beyond.

The modular approach sees each step in the analytical procedure as a separate module, from sampling (step 1), via subsampling to produce laboratory samples (step 2), processing of subsamples to produce test portions (step 3), extraction of the analyte, e.g. DNA (step 4), to the final quantitative analysis to produce a measurement of the GMO content (step 5). In principle, at each module, a specific method or protocol is applied to a matrix to produce the matrix for the successive step in the procedure. If the method or protocol used is valid and fit for the matrix, it will produce a new valid matrix, i.e. a matrix that satisfies particular requirements for downstream use. The idea is that each method or protocol can be validated separately, and once validated, can be combined with other modules in a flexible manner. For example, a particular food matrix can be subject to a number of alternative extraction methods, and each may be able to deliver PCR quality and quantity of DNA. Similarly, several PCR methods can be used to determine the quantity of maize DNA (absolute number of genome equivalent copies) in a DNA extract, and several PCR methods can be used to determine the quantity of e.g. genetically modified Mon810 maize (absolute number of target sequence copies) in a DNA extract. Each of these methods can be validated alone. To validate a detection method for a new GMO it will be much cheaper to validate only the GMO specific PCR method, than to include in the validation study a DNA extraction method and a species specific PCR method. And once several alternative methods are available, the use of these becomes more flexible. Testing for several GM maize (Mon810, Bt11, GA21 and T25) could for example be done on a single plate on a real-time PCR machine, using only a single reference gene (e.g. Adh). Some potential limitations of the modular approach have been identified and are currently being further investigated.

Overview of the single-laboratory pre-validation studies

In the first validation study related to this project, eight extraction methods were tested in a single-laboratory pre-validation study for four different matrices (see table). Each method was tested in a transfer-laboratory, which was different from the developer laboratory. Two of the methods tested were selected for a collaborative trial.

Table: Summary of single-laboratory prevalidation studies of the WP5.

<i>Methods</i>	<i>Species</i>	<i>Approximate timing of the experimental work</i>	<i>Number of Labs</i>	<i>Number of Protocols</i>	<i>Outcome and/or status</i>
Lecithin; 2 methods	soy	2001; June - August	1	2	The yield was considered too low for a full collaborative trial.
Crude soya oil; 2 methods	soy	2001; June - August	1	2	The yield was considered too low for a full collaborative trial.
Sweet corn flour; 2 methods	maize	2001; June - August	1	2	One of the methods chosen for full collaborative trial.
Native corn starch; 2 methods	maize	2001; June - August	1	2	One of the methods chosen for full collaborative trial.

Overview of the multi-laboratory validation studies

The most common form of the validation during the project was a multi-laboratory validation study. The inclusion of 2 – 5 laboratories provides already a good estimate of the method performance in an inter-laboratory setting. Thus, the results are valuable for the GMO testing community and the results can be obtained quicker than in the case of a full collaborative trial. Concurrently, such multi-laboratory validations can be used as a pre-validation study to verify the method performance prior to a full collaborative trial. In this role they serve better than an estimate based on the results of a single laboratory. Because the full ring-trials are very expensive all the methods cannot undergo a full validation study in practise. The total costs of a full ring-trial easily exceed 100,000 EUR, and the time required for such a study is commonly 1 – 2 years. Through the multi-laboratory validation the project has been able to offer information about the method performance on a much broader scale than what would have been possible if the same resources would have been allocated for full validation studies. An over-view of the multi-laboratory studies carried out during the project is listed in the following pages.

Altogether, 20 method protocols have undergone multi-laboratory validation during this project. The extraction methods needed further optimisation. This was carried out with subsequent method testing. Eight different protocols were tested for reference gene systems to be used for maize (4 reference genes), soy (two reference genes) and rapeseed (two reference genes). Genomic DNA samples were used, and the unknown samples were tested at the concentrations of 4 – 80 ng/μl. An experimental design with blind quadruplicate samples at six different concentration levels was used. The overall method performance was satisfactory, and a full

collaborative study has been carried out with the protocols of the maize reference genes. The results of soy reference genes suffered from poor quality of reagents.

Ten quantitative real-time PCR protocols were tested for maize and soy events (Soy: GTS 40-3-2; Maize Mon810, GA21, Bt176, Bt11 and T25). Nine of the methods were event-specific and one construct-specific (Maize GA21). Genomic DNA samples were used, and the unknown samples covered GM-range between 0.1% and 2.0%. For maize, the GM copy numbers range between 38 and 9,174 and for soya between 91 and 22,222. An experimental design with blind quadruplicate samples at six different concentration levels was used. The method performance was good for maize GA21, Mon810, Bt11 and T25 methods. The results for soy protocols suffered most likely from poor reagents quality and the maize Bt176 protocol needs some further optimisation.

Table: Summary of multi-laboratory validation studies of the WP5.

<i>Methods</i>	<i>Species</i>	<i>Approximate timing of the experimental work</i>	<i>Number of Labs</i>	<i>Number of Protocols</i>	<i>Outcome and/or status</i>
Reference gene systems					
Maize: Four reference gene systems (ADH, Invertase, Zein and HMG)	Maize	2002; August – October	4	4	All the reference gene systems yielded appropriate results, and were selected for a full collaborative trial. Adh system performed slightly better than the others, and was chosen to be used as a reference gene with all the GMO-specific methods. A publication including the results from the testing of the reference gene systems with different maize varieties as well as their comparison in a multi-laboratory study is under preparation.
Soy: Lectin reference gene system adapted to LightCycler and ABI machines	Soy	2002; August – October	3	2	The method performance in the study was not as good as expected based on the in-house testing of the method. The reason may have been poor quality of the reagents used in the study, because the reference gene system seemed to work together with the GMO-specific system in another study.
Rapeseed: ACC1 and ACC2 reference gene systems	Rapeseed	2002; August – October; repeated in January 2003	5 (2)	2	In the first study, the reagents and DNA quality were very poor, and therefore the results did not reflect the true method performance. An additional study with two laboratories and purified DNA as well as new reagents was carried out during January 2003. Both reference gene systems yielded appropriate results, but the ACC1 system performed slightly better than ACC2.
Extraction methods					
Soy (protein and flour); primary and secondary method	soy	2002; September – December	3	2	The participants encountered some problems in the execution of the protocol. Thus, after the validation study the protocol was refined and tested by different project participants. The refined protocol can be

<i>Methods</i>	<i>Species</i>	<i>Approximate timing of the experimental work</i>	<i>Number of Labs</i>	<i>Number of Protocols</i>	<i>Outcome and/or status</i>
					considered for further validation studies.
Event-specific quantitative real-time PCR methods					
Event GTS 40-3-2 (RoundupReady® soya)	Soy	Dec 2002 – March 2003	3	3	The method performance in the study was not as good as expected based on the in-house testing of the method. The reason may have been poor quality of the reagents used in the study. However, in contrast to the reference gene validation (See above) the reference gene system was working appropriately but the GMO-specific system seems to have suffered from the poor reagents quality.
Maize Bt176	Maize	Dec 2002 – March 2003	3	1	The method needs further optimisation before it can be recommended for further use.
Maize Bt11	Maize	Dec 2002 – March 2003	3	1	The method performed well. The method is currently validated in a full collaborative trial carried out by the JRC in collaboration with INRA, NVI, Syngenta and ENGL (European Network of GMO Laboratories). The trial will be concluded in 2003.
Maize T25	Maize	Dec 2002 – March 2003	3	1	The method performed well in the validation study. The validation results suffered from an error made in the DNA quantitation of the original stock and therefore a systematic error in the concentrations of the unknown samples was introduced (through serial dilution). This was corrected, and the method was further tested to verify its performance. The method is currently in a full collaborative trial carried out by the JRC in collaboration with INRA, BayerCropScience and ENGL (European Network of GMO Laboratories)
Maize GA21	Maize	Dec 2002 – March 2003	3	1	The method is construct but not event-specific. It performed very well in the validation study. However, an event-specific method for GA21 is currently under validation by the JRC in collaboration with Monsanto and the ENGL. Therefore, no full collaborative trial is foreseen for this method at the moment.
Maize Mon810	Maize	Dec 2002 – March 2003	3	3	All methods performed satisfactorily. One of the methods is presently being validated under the shared responsibility of the JRC and BfR in Germany.

Overview of the full collaborative validation studies

Due to the delays in material availability, sequencing and subsequent method development the activities of the validation work package started with considerable delay. The main works took place during the last year of the project. Still, altogether six protocols have undergone a full collaborative trial during the project. Some of the methods developed within the project will be fully validated after the end of the project duration, because they are very much needed by the European GMO testing community. As an example, the full validation of the Bt11 event-specific method started in the end of the project in collaboration between the JRC, INRA, NVI, ENGL and Syngenta. This collaborative trial has been concluded in October 2003, and the results are currently being prepared for the publication. An annex describing the Bt11 method is also under preparation and will be submitted to CEN to be considered as a European standard. Furthermore, the full validation of the T25 event-specific method developed in the project has already started in collaboration between the JRC, INRA, BayerCropScience and ENGL. Both the Bt11 and T25 collaborative trials are aimed for regulatory compliance, i.e. to fulfil the obligations of the availability of a validated method as required for instance by the GM Food and Feed Regulation (EC 1829/2003).

Table: Summary of full collaborative trials of the WP5.

<i>Methods</i>	<i>Species</i>	<i>Approximate timing of the experimental work</i>	<i>Number of Labs</i>	<i>Number of Protocols</i>	<i>Outcome and/or status</i>
Extraction methods for maize starch and sweet maize flour	Maize	September 2002 – January 2003	12	2	<ul style="list-style-type: none"> - A total of 11 laboratories reported the results. - The method for starch extraction provided appropriate yields, and a publication about the results is under preparation. - Several problems were encountered in the protocol for sweet maize flour, and the protocol needs to be refined before it can be recommended for further use.
Maize: Four reference gene systems (ADH, Invertase, Zein and HMG)	Maize	2003; January – April	12	4	<ul style="list-style-type: none"> - All the reference gene systems yielded appropriate results. - A publication about the results is under preparation. - An annex describing the ADH system is under preparation and will be submitted to CEN to be considered as a European standard.

Other contributions from the WP5

The experience gained in this scale about validation studies is extraordinary. The effort both from the WP leader, who has coordinated and organised all studies, prepared and tested all the materials and carried out all the data analysis as well as from the participating laboratories has been very high and intensive. It is clear that the contributions are clearly over anything that has been directly financed by the project. The efforts and the motivation have arisen from the general enthusiasm and good spirit within the project and among its participants but also from the very large interest shown in the project and in the availability of validated methods by the European GMO testing community. In addition, the experience gained within this workpackage has been beneficial for the drafting of the European standards within the CEN, for the European Network of GMO Laboratories, which is drafting a document defining minimum performance criteria for validated methods to be accepted as fit for regulatory compliance (see <http://engl.jrc.it/>) and for the JRC as it is preparing to become the European Community Reference Laboratory for GMO testing with the main task of method validation (established in the Regulation EC 1829/2003).

Scientific publications with contributions from WP5 (peer reviewed journals, books, etc.):

1. Holst-Jensen, A., S.B. Rønning, A. Løvseth & K.G. Berdal. 2003. PCR technology for screening and quantification of genetically modified organisms (GMOs). *Anal. Bioanal. Chem.* 375: 985-993.
2. Holst-Jensen, A. 2003. Advanced DNA based detection techniques for genetically modified food. Chapter 27. *In: Food Authenticity and Traceability.* M. Lees (ed.), Woodhead publishing, UK, ISBN 1 85573 526 1.
3. Holst-Jensen, A. & K.G. Berdal. 2003. The modular analytical procedure and validation approach and the units of measurement for genetically modified materials in foods and feeds. *J. AOAC Int.* (revised version submitted).
4. Several authors 2003. Testing and multi-laboratory comparison of maize reference genes *adh*, *zein*, *invertase* and *hmg*. In preparation.
5. CEN/ISO 2003. Target taxon specific method for the absolute quantitation of the *adh1* gene DNA of maize using real-time PCR. Annex proposed to prEN ISO 21570.
6. CEN/ISO 2003. Target taxon specific method for the absolute quantitation of the *lec1* gene DNA of soyabean using real-time PCR. Annex proposed to prEN ISO 21570.
7. CEN/ISO 2003. Target taxon specific method for the absolute quantitation of the *BnACCg8* gene DNA of rapeseed (canola) using real-time PCR. Annex proposed to prEN ISO 21570.
8. CEN/ISO 2003. Event specific method for the absolute and relative quantitation of DNA from genetically modified Bt11 maize using real-time PCR. Annex proposed to prEN ISO 21570.
9. CEN/ISO 2003. Event specific method for the absolute and relative quantitation of DNA from genetically modified GTS 40-3-2 soyabean using real-time PCR. Annex proposed to prEN ISO 21570.
10. Several authors. Validation of maize reference genes *adh*, *zein*, *invertase* and *hmg*. In preparation.
11. Several authors. Validation of a DNA extraction method for maize starch. In preparation.
12. Several authors. Multi-laboratory validation of nine event-specific quantitative methods – results and lessons. In preparation.

Oral and poster presentations from WP5 at scientific conferences, etc.:

1. Holst-Jensen, A. (2001). Development of standardised detection and quantitation methods for genetically modified foods. Poster presented at the conference “The Biotech society – where are we going?” organised by the Norwegian Research Council, Oslo, June 5th 2001.
2. Holst-Jensen, A. (2002). PCR technology for screening and quantification of GMs. Oral presentation at Euroanalysis 12, Dortmund, Germany, September 9-13th 2002.
3. Holst-Jensen, A. (2002). Detection, identification and quantification of GMOs and derived products: *present and future challenges*. Oral presentation at the ENGL stakeholders meeting, Brussels, December 3rd 2002.
4. Puumalainen, J. (2003). Methodvalidierung am JRC. Oral presentation in the workshop “Gentechnisch veränderte Organismen – Qualitätssicherung in der Analytik”. Wien, Austria, 18 March, 2003.

5. Holst-Jensen, A. (2003). Modular validation of the analytical procedure for food/feed analysis including GMO testing. Oral presentation at the 2nd ENGL plenary meeting, Ispra, Italy, April 2nd, 2003.
6. Puumalainen, J., Paoletti, C. & Van den Eede, G. (2003). Sampling and testing. Latest news from the JRC. Invited oral presentation in the annual meeting of the European Enforcement Group for deliberate release and contained use of GMOs. Thun, Switzerland, 7 – 8 May, 2003.
7. Berdal, K.G. & Holst-Jensen, A. (2003). Detection, traceability, and quality assurance, oral presentation at the ENTRANSFOOD concluding conference, Rome 29-30 May, 2003.
8. Puumalainen, J. (2003). Introduction into method validation. Invited oral presentation in the annual meeting of the EU shared cost action project GMOChips. Barcelona, Spain, 5 – 6 June, 2003.
9. Holst-Jensen, A. (2003). Products derived from genetically modified organisms (GMOs) – Reference materials for method development and validation. Oral presentation at the Ninth International Symposium on Biological and Environmental Reference Materials, Berlin, Germany, June 15-19th, 2003.
10. Puumalainen, J. & Van den Eede, G. (2003). Method validation in the context of the European Network of GMO Laboratories. Invited speaker at the IUPAC (International Union of Pure and Applied chemistry) World Conference in Ottawa, Canada, 10 – 15 August, 2003.
11. Puumalainen, J. & Van den Eede, G. (2003). Aims and requirements of method validation – examples of detecting GMOs in food. Invited speaker at the Biotech 2003 (7th Biotech at Crossroads Conference), Nantes, France, 25-26 September, 2003.

Other references related to the WP5 (peer reviewed journals, books):

1. Anklam, E., P. Heinze, S. Kay, G. Van den Eede and B. Pöpping. 2002. Validation studies and proficiency testing of methods for the detection and quantification of Genetically Modified Organisms (GMO's). J. AOAC Int., Vol. 85, No. 3, 2002 pp. 809-816.
2. Bonfini L., S. Kay, H. Petra and G. Van den Eede. 2002. Report on GMO detection identification and quantification methods submitted to collaborative studies. EUR 20383 EN (2002). Available in <http://biotech.jrc.it/docs.htm>.
3. Bonfini L., S. Kay, H. Petra and G. Van den Eede. 2002. Review of GMO detection and quantification techniques. EUR 20384 EN (2002). Available in <http://biotech.jrc.it/docs.htm>.
4. Van den Eede, G., H. Schimmel, S. Kay and E. Anklam. 2002. Analytical Challenges: Bridging the gap from regulation to enforcement. J. AOAC Int., Vol. 85, No. 3, pp. 757 - 761.
5. Bonfini, L & Van den Eede, G. 2002. Detection and Quantification of GMOs. In Bock, Lheureux, Libeau-Dulos, Nilsagård & Rodriguez-Cerezo (eds.) "Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture". P: 75-89. Available in http://www.jrc.cec.eu.int/download/GMCrops_coexistence.pdf.
6. ENGL 2003. Definition of minimum performance requirements for analytical methods of GMO testing. Draft document. 23 p.