Asia Pacific Metrology Program (APMP) - Asia Pacific Accreditation Cooperation (APAC) Joint Proficiency Testing Programme

< APAC T111 >

Event-specific quantitative analysis for genetically modified (GM) Maize Line MON87427

Protocol

Coordinated by

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National Institute of Metrology, China (NIM)

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- **Introduction**

Conventional breeding of crop varieties cannot meet the food supply demands of the increasing world population. To fulfill the needs, agricultural biotechnology has developed various genetically modified (GM) crops for insect or herbicide resistance, abiotic stress tolerance, or nutritional improvement. Although, there is a scientific consensus that currently available food derived from GM crops poses no greater risk to human health than conventional food, each GM food needs to be tested on a case-by-case basis before introduction. Nonetheless, members of the public are much less likely than scientists to perceive GM foods as safe. Therefore, it is of important practical significance to detect GM crops from different trading sites.

Labeling of GMO products in the marketplace is required in 64 countries.[1] Labeling can be mandatory up to a threshold GM content level (which varies between countries) or voluntary. In Canada and the USA labeling of GM food is voluntary,[2] while in Europe all food (including processed food) or feed which contains greater than 0.9% of approved GMOs must be labelled.[3] Japan, Malaysia, New Zealand, and Australia also require labeling so consumers can exercise choice between foods that have genetically modified, conventional or organic origins.[4]

The Cartagena Protocol sets the requirements for the international trade of GMOs between countries that are signatories to it. Any shipments containing genetically modified organisms that are intended to be used as feed, food or for processing must be identified and a list of the transgenic events be available.

Maize is the most widely grown grain crop throughout the world. Specific maize strains have been genetically engineered to express agriculturally-desirable traits, including resistance to pests and to herbicides. Maize strains with both traits are now in use in multiple countries. GM maize MON87427 has the characteristics of glufosinate herbicide tolerance and is one of the most commonly used strains in processed food and feed.

With increasing international trade of GM crops and processed agricultural products, traceable measurements of GMO contents have become one of the essential requirements for ensuring food safety and meeting trading requirements.

With the aim of enhancing the measurement capability and traceability of

measurements in countries and economies of the Asia-Pacific region through a better regional scientific infrastructure, the Asia-Pacific Metrology Programme (APMP) and the Asia Pacific Laboratory Accreditation Cooperation (APLAC) has agreed to strengthen cooperation between the two organizations. At the 2013 APMP General Assembly and Related Meetings, APMP and APLAC agreed to establish the APMP-APLAC Joint Proficiency Testing Working Group (PTWG) as a formal infrastructure to provide more proficiency testing (PT) programmes with metrologically traceable reference values for performance evaluation purpose for participating measurement institutes and laboratories in the region. Upon the merging of APLAC with Pacific Accreditation Cooperation, the Asia Pacific Accreditation Cooperation (APAC) was established on 1 January 2019.

At the 2018 meetings, National Institute of Metrology (NIM), China proposed a new joint PT study on the determination of event-specific quantitative analysis for genetically modified (GM) maize line T25 for 2019 to the APMP-APAC Joint PT Working Group and the Technical Committee on Amount of Substance (TCQM). The purpose of this study is to demonstrate the capability of participating laboratories in measuring event-specific quantitative analysis for genetically modified (GM) maize.

During prepared more proficiency testing samples, T25 candidate materials were examined and found that the positive samples had severe mildew. The re-preliminary T25 samples were tested by real-time quantitative PCR and digital PCR. The results of the two methods were inconsistent.

100 % pure GM (MON87427) maize and 100 % pure non-GM(MON87427) maize was been tested and found is suit to prepare the proficiency testing samples. The preliminary MON87427samples were tested by real-time quantitative PCR and digital PCR. The results of the two methods were consistent. So, at the 2020 meeting, National Institute of Metrology (NIM), China **suggested to replace the sample for proficiency testing from T25 to MON87427.**

Another, the short-term stability testing has been done by NIM from May 14,2020 to June 4 ,2020 for the proficiency testing sample of MON87427. The samples were thought to be stable when kept at 25℃、37℃、60℃ in a vacuum packaging with tin foil paper. The minimum sample size was 100 mg, and DNA was extracted by plant genomic DNA Extraction Kit (product No. dp305, Tiangen Biotechnology Co., Ltd.). Using the extracted genomic DNA as template, the specific sequence and internal standard gene of mon87427 transformant were amplified by mon87427 / zssiib duplex digital method. The copy number of mon87427 transformant and internal standard gene was measured, and the copy number ratio of mon87427 transformant and internal standard gene was calculated. The results show that the difference of characteristic value changes is not significant. After 21 days of storage at 60 ℃ for 21 days, the change trend of transgenic content was not significant, which indicated that the quality value of samples was stable even in summer.

The reference values provided by NIM will be used as the assigned values for evaluating the performance of the participants in this PT programme. The relevant Calibration and Measurement Capabilities (CMCs) of NIM on GMOs in food are registered in the BIPM Key Comparison Database (KCDB). The use of reference values as the assigned values for this PT programme will allow more rigorous evaluation of the accuracy of participants' results. It will not only enhance the quality of the PT programme but also help build the measurement capabilities of the participants through a better linkage between the NMIs/DIs and the analytical laboratories in the Asia-Pacific region.

- **Objectives**

The aim of this study is to demonstrate the capability of participant's real time PCR or digital PCR capabilities in determining GM target gene contents including the GM gene and the endogenous gene.

- **Organisers of the joint PT Programme**

NIM (Address: No.18, Bei San Huan Dong Lu, Chaoyang Dist, Beijing, P.R.China) is the National Measurement Institute for China, under the Mutual Recognition Arrangement of the International Committee for Weights and Measures (CIPM MRA). NIM is the provider of this proficiency testing programme and takes responsibility for all tasks in the development and operation of the proficiency testing programme, including preparation and distribution of proficiency test samples, data analysis and evaluation of results, preparation of interim and final reports, and communications with participants. ISO/IEC 17043:2010 [5] will be followed.

- **Fee for participation**

Free of charge.

- **Call for participation**

APAC members and APMP Developing Economies' Committee (APMP DEC) members will be invited to participate in this program. Once this proposal is approved by the APAC Technical Committee, invitations will be sent to all APAC members through their accreditation bodies, and to APMP DEC and TCQM members by both APMP DEC and TCQM Chairs.

Total number of participants for this Joint PT programme will be 100.
Laboratories nominated by the APMP DEC are about 30, including TCQM members.

Laboratories nominated by APAC accreditation bodies and non-APAC accreditation bodies are about 70.

The participation will be confirmed within two weeks after the deadline of the nomination with the assigned laboratory codes.

- **Test sample**

GM maize MON87427 has the characteristics of glufosinate herbicide tolerance and is one of the most commonly used strains in processed food and feed. Unknown samples 1 and 2 were prepared by mixing dried powders of 100 % non-GM maize （EXP262） and 100 % GM maize (MON87427) and subsequently homogenizing the mixtures. The 100 % GM maize (MON87427) will be used to prepare the standard curve for the real-time PCR. Both GM maize unknown samples to be analyzed are powders containing a low but different content of GM maize. The GM content is the copy number ratio between the GM gene and the endogenous gene will be detected ranges between 1/1000 to 1/10. The powder of 100 % pure GM (MON87427) maize, and 100 % pure non-GM maize, and two GM maize samples each with an unknown GM content have been made by NIM. GM maize powders (MON87427) and non-GM maize powders have been provided by the Development Center for Science and Technology, Ministry of Agriculture and Rural Affairs. The purity of GM maize have been tested by the Oil Crops Research Institute Chinese Academy of Agricultural Sciences，and the Biotechnology Research Institute, Chinese Academy of Agricultural Sciences.

Homogeneity analysis of the 2 unknown samples were performed at NIM, where 11 vials were analyzed using a QRT-PCR and digital PCR method. The sample intake used for determining the homogeneity was 100 mg. Stability testing has been done by NIM since Mar 2th， 2019. Samples were packed in vacuum-sealed foil bags. Long term storage of the material at NIM is kept under 4°C in a vacuumed-desiccator.

Sample list:

- Powder of 100 % pure GM (MON87427) maize, 1 unit, 1g/unit;
- Powder of 100 % pure non-GM maize, 1 unit, 1g/unit;
- Powder of Unknown1 GM content prepared with 100 % pure GM (MON87427) maize and 100 % pure non-GM maize; 3 units, 1g/unit;
- Powder of Unknown2 GM content prepared with 100 % pure GM (MON87427) maize and 100 % pure non-GM maize; 3 units, 1g/unit;

Primers and Probes (European Union Reference Laboratory for GM Food and Feed Validated Method Maize MON87427)

Name Oligonucleotide DNA Sequence (5′ to 3′)

MON87427 target sequence

MON87427 5’ -ACGGAAACGGTCGGGTCAAATG-3’

MON87427 5’ -CCATGTAGATTTCCCGGTTTTCTC-3’

MON87427 (Probe) FAM-5’-TCGGGACAATATGGAGAAAAAGAAAGAG -3’-BHQ1

Reference gene zSSIIb target sequence

zSSIIb 5′-CGGTGGATGCTAAGGCTGATG-3′

zSSIIb 5′-AAAGGGCCAGGTTCATTATCCTC-3′

zSSIIb (Probe) Hex-5′-TAAGGAGCACTCGCCGCCGCATCTG-3′-BHQ1

Reaction system of digital PCR for MON87427 and zSSIIb

Chart 1 System of digital PCR reaction for MON87427 and zSSIIb

Regent	Volume (μL)	notes
DNA Template	1	40 ng/μL
zSSIIb-F (10 μM)	0.8	
zSSIIb-R (10 μM)	0.8	
zSSIIb-P (10 μM)	0.4	
MON87427-F (10 μM)	0.8	
MON87427-R (10 μM)	0.8	
MON87427-P (10 μM)	0.4	
ddPCR Mix（2×）	10	
ddH2O	5	

Thermal cycling procedure of digital PCR

Chart 2 Thermal cycling procedure of digital PCR for MON87427 and zSSIIb

Stage and Step	Time/Temperature	**cycling**
1	10 min/94°C	1
2	30 s/94°C	40
3	1 min/ 60.5°C	
4	10 min/ 98°C	1
5	4°C	1

Reaction system of real time PCR for MON87427 and zSSIIb

Chart3 **Reaction system of real time PCR** for MON87427 and zSSIIb

Regent	Volume (μL)	notes
2× Mix	12.5 μL	The DNA which concentration is 100ng/μL, 50ng/μL, 25ng/μL,10ng/μL,5ng/μL,1ng/μL from 100 % pure GM (MON87427) maize will be the standard for real time PCR. The concentration of unknown is about 25ng/μL.
Primer-F(10 μM)	0.8μL	
Primer-R(10 μM)	0.8μL	
Probe-P(10 μM)	0.4 μL	
DNA Template	2 μL	
ddH2O	8.5 μL	
Total	25 μL	

Thermal cycling procedure of real time PCR

Chart 4 Thermal cycling procedure of real time PCR for MON87427 and zSSIIb

Stage and Step	Time/Temperature	cycling
1	3min/95°C	1
2	10 s/95°C	45
3	1 min/ 60°C (data collection)	
4	4°C	1

Reporting and submission of results

Participants should complete the Result Report. The instructions on the manner of reporting test results are as follows:

- Units of measurement: Report the copy number ratios between the GM gene and the endogenous gene of the analytes and associated uncertainties;
- Number of significant figures: Report the test results to 2 significant digits;
- For each analyte, the mean value of at least three independent measurements, the expanded uncertainty of the mean value and the coverage factor (which gives a level of confidence of approximately 95 %) should be reported;
- Participants should provide information on the methods of analysis;

Participants should be aware that any submitted results are considered final, and results and units should be thoroughly checked before submission. Participants should submit the Result Report electronically to the coordinator of the PT programme before the deadline. Results submitted after the deadline will not be accepted. Participants are reminded that the ability to report results in the specified unit and within the given time are part of the requirements of the proficiency test.

- **Measurement uncertainty**

Measurement uncertainty is best estimated within the individual laboratory

environment. An estimate of measurement uncertainty is normally based on the combination of a number of influencing parameters (components of uncertainty). As stipulated in ISO Guide to the Expression of Uncertainty in Measurement [7], the uncertainty of each individual parameter should be quantified and expressed numerically as a standard uncertainty. These values are then combined according to the rules of the propagation of uncertainty and the combined standard uncertainty is multiplied by a coverage factor to produce an expanded uncertainty at the 95 % level of confidence.

- **Evaluation of performance of participants**

The performance of the participating laboratories will be assessed using z-score, which is calculated as follows [5][6]:

$$z_i = \frac{x_i - x_{pt}}{\sigma_{pt}}$$

Where x_i : the participant's result

x_{pt} : the assigned value*

σ_{pt} : the standard deviation for proficiency assessment estimated from the Horwitz equation

> * Note: The reference values determined by NIM will be used as the assigned values which are based on the CMCs claims of NIM listed on the Appendix C of the Key Comparison Database (KCDB). This is in accordance with the ISO/IEC 17043 recommendations on the determination of assigned values for proficiency testing schemes [5].

z-Score is commonly interpreted as:

(i)	$z \leq 2.0$	Satisfactory
(ii)	$2.0 < z < 3.0$	Questionable
(iii)	$z \geq 3.0$	Unsatisfactory

Laboratories having a |z| score equal to or larger than 3.0 shall thoroughly investigate their results for the discrepancy and those having a z-score in the range $2.0 < z < 3.0$ are also encouraged to review their results.

For reference purpose, the performance of the participating laboratories will be assessed using zeta-score (ζ), which is calculated as follows [6]:

$$\zeta_i = \frac{x_i - x_{pt}}{\sqrt{u^2(x_i) + u^2(x_{pt})}}$$

Where x_i:the participant's result

x_{pt}: the assigned value (* note above)

$u_{(xi)}$:the participant's own estimate of the standard uncertainty of its result x_i.

$u_{(xpt)}$:the standard uncertainty of the assigned value x_{pt}

ζ-scores are interpreted as in the same way as z-scores using the same critical values of 2.0 and 3.0. ζ-scores may be used in conjunction with z-scores, as an aid for improving the performance of laboratories as follows. If a laboratory obtains |z| scores that exceed 3.0, they may find it of value to examine their test procedure step by step and derive an uncertainty budget for that procedure. The uncertainty budget will identify the steps in the procedure where the largest uncertainties arise, so that the laboratory can see where to expend effort to achieve an improvement. If their |ζ| scores also exceed the critical value of 3.0, it implies that their uncertainty budget does not include all significant sources of uncertainty[6]. Laboratories are encouraged to review their uncertainty budget.

- **Issue of reports**

An interim report will be issued to the participants for checking of transcription errors made by the PT provider. The draft final report will be then prepared and submitted to the Joint PTWG under the collaboration of APMP and APAC for comments and recommendations to the APAC Technical Committee, which will approve the Final Report for publication after its review. An electronic copy of the Final Report will be distributed to the participants and their respective accreditation bodies.

- **Programme schedule (proposed)**

The proposed time schedule for the various phases of the proficiency testing programme is as follows:

Time schedule

Calling for participation: Feb 2021
Deadline for registration: April 10th , 2021
Sample distribution from NIM: June, 2021
Deadline for reporting July 30th, 2021
Discussing the result: APMP-APAC Meeting, 2021
The draft final report: March 2022

- **Confidentiality**

The concerned parties (APMP, APAC and NIM) strive to maintain strict

confidentiality of the characteristic properties of the proficiency test samples distributed and the performance of all participating laboratories. To preserve the confidentiality, participants receive reports giving all results for assessment but without identifying individual laboratories. The code number assigned to a participant in the proficiency testing programme will be provided only to the contact person/authorized person of the participating laboratory and the respective accreditation body.

- **Contact**

Contact information

This joint PT is coordinated by National Institute of Metrology, China

For enquiries, participants may wish to make contacts as follows:

Gao Yunhua, NIM, gaoyh@nim.ac.cn

- **References**

[1] Hallenbeck, Terri (2014-04-27). "How GMO labeling came to pass in Vermont". Burlington Free Press. Retrieved 2014-05-28.
[2] Botha, Gerda M.; Viljoen, Christopher D. (2009). "South Africa: A case study for voluntary GM labelling". Food Chemistry. 112 (4): 106064. doi:10.1016/j. foodchem.2008.06.050.
[3] John Davison (2010)"GM plants: Science, politics and EC regulations" Plant Science 178(2):94–98.
[4] Northwestern Journal of Technology and Intellectual Property Paper on: "Consumer Protection" Consumer Strategies and the European Market in Genetically Modified Foods
[5] ISO/IEC 17043:2010 “Conformity assessment – General requirements for proficiency testing”, 2010, Geneva, Switzerland.
[6] ISO 13528:2015 “Statistical methods for use in proficiency testing by interlaboratory comparison”, 2015, Geneva, Switzerland.
[7] ISO/IEC Guide 98-3:2008 “Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)”, 2008, Geneva, Switzerland.