HOKLAS Supplementary Criteria No. 21

'Chemical Testing' and 'Food' – Polymerase Chain Reaction (PCR) Testing

0 Introduction

(a) This document serves to clarify and supplement the requirements of ISO/IEC 17025:2017 and HKAS Policy Documents No. 1 for the accreditation of laboratories performing (i) polymerase chain reaction (PCR) tests for gene doping detection under Test Category of 'Chemical Testing'; and (ii) detection and quantification of genetically modified organisms (GMO) under Test Category of 'Food'.

(b) Laboratories should note that fulfilling the requirements in this document might not necessarily meet the requirements of test standards. Individual test standards may have specific requirements, which shall be met when conducting the tests.

1 Scope

(No additional explanation)

2 Normative references

(No additional explanation)

3 Terms and definitions

(No additional explanation)

4 General requirements

(No additional explanation)

5 Structural requirements

5.1 The laboratory shall have at least one staff member who possesses adequate knowledge of and experience in PCR testing. He/she shall be responsible for the technical operation with respect to PCR testing in the laboratory.

6 Resource requirements

6.1 General

(No additional explanation)

6.2 Personnel

(a) The minimum level of qualification and experience necessary for all technical personnel of the laboratory shall be defined and shall be appropriate to the duties and responsibilities of the staff members.

(b) Testing shall be performed by staff members who have undergone formal training in PCR testing. A training programme shall include, in addition to the test procedures, knowledge in sample collection, training in sample handling, prevention of cross contamination, data handling and quality control techniques. Training materials should be documented and authorised. Full records of training shall be maintained.

(c) Approved signatories

(i) Approved signatories shall have as a minimum a bachelor degree in biology, biochemistry, chemistry or equivalent and possess relevant testing experience of not less than three years. Appropriate membership of professional bodies is also acceptable in lieu of academic qualifications. In addition, he/she shall have at least six months experience in the areas of testing for which signatory approval is sought.

(ii) Special consideration may be given to persons without the above qualifications but with extensive experience (i.e. at least 10 years) in the test area concerned.

(iii) In all cases, candidates shall demonstrate to the assessors his/her technical competence in the test area under consideration before signatory approval can be granted.

6.3 Facilities and environmental conditions

(a) The laboratory should be clean and have adequate lighting at bench tops. Temperature and humidity shall be controlled and their acceptable ranges shall be defined. There shall be effective separation of the PCR testing area from neighbouring laboratory areas to minimise the spread of contamination from nucleic acids and/or nuclease (both DNase and RNase). Since minor degrees of cross-contamination would result in erroneous results by nucleic acid amplification, a distinct room shall be used for PCR testing in a laboratory complex. Contamination in major testing areas shall be regularly monitored (e.g. by analysing 'open-tube' blanks or bench surface swabs) and related records shall be maintained. Appropriate corrective actions shall be taken if contamination is detected. Prevention of contamination is also essential. Procedures and precautions taken in avoiding cross-contamination shall be documented. Such procedures shall include washing of labware, generation of distilled, deionised or reagent water, decontamination of equipment between samples during PCR analysis, cleaning of work surfaces and other relevant activities.

(b) Where the areas for preparation of reagents and samples are located within a single room, distinct separation of these activities shall be maintained and appropriate procedures and control shall be adopted to prevent cross-contamination.

(c) Reagents, consumables and equipment shall be located at appropriate designated areas to serve their specific purposes. Nucleic acid samples should be stored in designated refrigerated compartments after sample preparation. They shall not be kept at areas where activity such as gel electrophoresis or PCR work is conducted.

(d) At least 4 separate rooms or clearly designated areas shall be provided for the following processes:

- sample receipt;
- sample preparation and extraction;
- reagents preparation and dispensing of master mix; and
- amplification and product detection.

(e) Separate enclosures shall be provided for the storage of the following materials:

- certified reference materials;

- negative PCR control;
- positive PCR control/plasmid/vector;
- test samples;
- sample extracts;
- kits, master mix, DNA polymerase, primers, probes, reagents;
- DNA extracts after sample preparation; and
- PCR products.

(f) The movement of nucleic acid samples or specimens shall be unidirectional (i.e. from pre-amplification to post-amplification areas). Arrangement shall be made to ensure that any materials (e.g. printouts, stationary, etc.) in/from post-amplification areas are protected from cross-contamination.

6.4 Equipment

(a) Commonly used equipment for PCR tests includes balances, thermometers, pH meter, micropipettes, timer, centrifuges, volumetric labware. Performance and precision of these items of equipment shall meet the specifications of the tests. The requirements relating to maintenance, verifications and calibrations of these items of equipment described in HOKLAS SC-02 shall apply.

(b) Automated liquid handling system for DNA extraction, quantification and/or PCR set-up shall be verified regularly to ensure that their performance, including accuracy and precision, complies with the specifications of the tests. The requirements relating to maintenance, verifications and calibrations of volumetric apparatus described in HOKLAS SC-02, if applicable to the tests performed, shall apply.

(c) The performance of the thermocycler and its built-in optical spectroscopic components shall be verified regularly to ensure that the tolerances required by test standards are complied with. Laboratories shall ensure that different equipment used for different testing purposes shall be subjected to appropriate scheduled calibration and performance checks covering all critical parameters such as temperature and time that have an effect on the validity of test results. Temperature and optical signal at all reaction wells shall also be verified. These calibrations and checks should be performed at least once a year, or at the frequency recommended by the manufacturer, whichever is more frequent.

(d) Micropipettes shall be designed to prevent the possibility of aerosol production. Aerosol resistant pipette tips or positive displacement pipettes are strongly recommended. General instruments such as vortex mixers and micro-centrifuges should be placed at as great a distance from sample preparation work place as possible. Thermocycler shall be placed at post-amplification areas to prevent cross contamination.

6.5 Metrological traceability

(a) General requirements for reference materials

(i) Laboratories shall demonstrate traceability by use of certified reference materials obtained from recognized national metrology institutes such as the Institute for Reference Materials and Measurements (IRMM) as far as possible.

(ii) Nucleic acids extracted from reference materials are stored to provide reference stocks. Reference stocks shall be stored at a condition to minimise nucleic acid degradation. Laboratories shall have a policy and procedures for purchase, handling, storage, maintenance and use of reference materials and stocks.

(iii) Reference stocks should be aliquoted to minimise degradation due to freezing and thawing. Laboratories should verify stability of stock DNA. Procedures for verification of stocks should be documented.

(iv) The following records shall be maintained:

- the sources, lot numbers, dates of receipt and expiration, dates put in use, conditions and integrity of packaging of reference material;
- preparation records of reference stocks with dates of preparation, expiration, and name of operator;
- verification records of reference stocks; and
- records of monitoring of environmental conditions for storage of reference stocks.

(b) Specific requirements for reference materials for GMO testing

(i) Positive DNA reference materials/plasmids/vectors obtained from sources other than national metrology institutes shall be verified by

counter checking with at least one reference material from a different manufacturer source, if available, before use.

(c) Specific requirements for reference materials for gene doping detection

(i) When certified reference materials are not available, non-certified positive DNA reference materials/plasmids/vectors shall be sequenced and verified by homology search with reference DNA sequences in public genetic sequence databases (e.g. National Center for Biotechnology Information (NCBI) with nucleotide collection consisting of GenBank+EMBL+DDBJ+PDB+RefSeq sequences) before use. The matched reference DNA sequences in the database shall be independently submitted by at least two different groups of researchers and the homology between the matched reference DNA sequences shall be 100%.

(ii) If another independent source of reference DNA sequence is not available in database (i.e. the reference DNA sequence is submitted by a single group of researchers only), the following requirements shall be fulfilled:

(1) The method used for obtaining the reference DNA sequence in database shall be based on Sanger sequencing.

Note: Methods based on Next-Generation Sequencing (NGS) may be used provided that the sequencing quality shall reach a base call accuracy of 99.9% or above, which is equivalent to Phred Quality Score 30 (i.e. Q30) or above.

(2) Laboratory shall demonstrate the authenticity of the reference DNA sequence in database through proper verifications (e.g. in-silico matching of cDNA obtained from reverse transcription of blank sample by Sanger or Next-Generation Sequencing, bioinformatics from whole genome sequencing.).

Note: Alternatively, it is also acceptable if the reference sequence has been curated by NCBI Reference Sequence Database (RefSeq) with a status of 'REVIEWED'.

(3) The laboratory shall have a documented mechanism for periodic

review of the presence of new reference database sequence from an independent source, at least on a quarterly basis.

(iii) To infer homology, DNA sequences/genetic patterns of the non-certified nucleic acid material shall match with those of the reference DNA sequence(s) in the genetic sequence database at 100%, and the match shall cover 100% of the sequence of non-certified nucleic acid material.

(iv) Quantities with the character of a count including copy number concentration of a particular nucleic acid sequence shall be verified by primary reference measurement procedures such as digital PCR or appropriate internationally recognized quantification techniques (e.g. spectrophotometry, fluorometry) and/or counter checking with at least one reference material from a different manufacturer source, if available, before use.

6.6 Externally provided products and services

(a) Chemicals and reagents used in the process from sample preparation to PCR testing shall be of molecular biology grade or equivalent and they shall be free from contaminating nucleic acids and/or nuclease (for both DNase and RNase). Extraction buffer or solution has to be autoclaved prior to use except for nucleic acid and nuclease free ready-to-use buffers. Any special precautions in preparation or use of the reagents, including relevant information on the stability of reagents to heat, air, light and other chemicals, etc, shall be documented. Reagents prepared in laboratory should be labelled with the identity, strength, types of solvent used and dates of preparation and/or expiration. Any special precautions, hazards or restrictions in using the reagents shall also be indicated on the labels. Personnel responsible for preparation of reagents shall be identifiable from records.

(b) The sources and histories of consumables having an effect on the validity of test results, such as DNA polymerase, shall be documented. Records on information such as supplier, lot number, date received, date put in use, date of verification and date of expiration of all critical materials shall be maintained.

(c) DNA polymerase/master mix/kits/primers and probes

All DNA polymerase/master mix/kits/primers and probes shall be checked for their physical conditions and verified for their performance for every new batch prior to release for use. Verification procedures, criteria for acceptance, shelf lives, and special storage conditions shall be documented. Records of verification and monitoring of the storage conditions shall be maintained.

(d) Primer sets / Primer-probe sets
Evidence showing the nature or sequence of the primer and/or primer-probe set shall be provided. The primer shall be verified for their performance using target DNA positive material.

(e) The software and/or computer programmes used for data analysis shall be verified and recorded.

7 Process requirements

7.1 Review of requests, tenders and contracts

(No additional explanation)

7.2 Selection, verification and validation of methods

7.2.1 Selection and verification of methods

(a) Laboratories shall preferably use national, regional, and international standard methods, or standard methods published by reputable professional bodies. Laboratories may also use laboratory-developed methods but they have to be validated. Laboratories shall demonstrate that each particular method is adequate for its intended purpose and the needs of the customers are met. When standard methods, or methods that have been validated by collaborative studies (including proprietary test kits or systems) are used, laboratories shall verify their own ability to achieve satisfactory performance against the documented performance characteristics of the method by use of certified reference materials or participation in relevant proficiency testing programmes. Laboratories shall pay attention to the limitations, concentration range and applicable sample matrices specified in the test standards. Standard test methods that are used outside their scope of application shall be validated. For guidance on method verification for GMO and gene doping testing, laboratories may refer to ‘Verification of

analytical methods for GMO testing when implementing interlaboratory validated methods' published by the Joint Research Centre of the European Commission and ISO 20395 'Biotechnology – Requirements for evaluating the performance of quantification methods for nucleic acid target sequences – qPCR and dPCR' respectively.

7.2.2 Validation of methods

I <u>For detection and quantification of GMO by PCR</u>

(a) Test methods

There are several types of PCR testing for detection and quantification of GMO, including, *inter alia*, qualitative, semi-quantitative and real-time quantitative tests. Requirements for method validation for different types of testing vary slightly.

(i) GMO test methods shall include information on the target GM events together with the traits being tested for (e.g. clearly identifying which traits contain 35S and/or nopaline synthase (nos)) and other GM materials (crops) which are available on the market, so as to avoid inappropriate testing and inappropriate claims made from results. Specificity of the method shall be demonstrated for the target GM events and/or the taxon specific target sequences.

(ii) When a GMO screening test (such as for 35S promoter, nos terminator, etc.) is used as a preliminary detection tool, it shall be validated to demonstrate that it would detect a defined range of individual GM traits since the limits of detection may vary between traits.

(iii) If a GMO screening test is negative and no further testing is being conducted, the result shall be reported as no GM material detected, with a specification of which traits have been excluded.

(iv) If a GMO screening test is positive, the laboratory shall proceed to determine the specific trait present. However, if the specific trait is not found, the laboratory shall specify the range of traits that may be present, and state clearly that the positive results cannot be verified without further determination. Contamination by cauliflower mosaic virus DNA (35S) is unlikely, but the potential for

contamination by *Agrobacterium* bacteria (*nos*) from soil is greater. Unless a specific trait is determined, the possibility remains that the positive screening test is due to contamination. Another possibility is that an unknown and/ or unexpected trait is present.

(b) Method validation

(i) Laboratories shall document clearly those matrices which are not suitable for testing. For instance, owing to the absence of DNA, matrices such as refined oils shall not be tested.

(ii) There are some processed food matrices (e.g. soy sauce) where the integrity of the DNA needs to be assessed to decide whether the test has any validity.

(iii) The laboratory shall also document clearly the matrices which are suitable for quantification. Quantification based on a reference material of a given matrix may not be appropriate for the same traits in a different (e.g. processed) matrix.

(iv) As the availability of GM reference materials for quantification will always lag behind the traits that are on the market, the laboratory may mix its own quantification standards from 100% GM material, provided that the purity of the materials (GM and non-GM) is established, and proper validation has been undertaken.

(v) Laboratories shall determine the method performance characteristics including limits of detection, limits of quantification, dynamic range, amplification efficiency, bias, precision and robustness, for in-house developed tests and tests based on modified standard methods, wherever applicable. Laboratories may refer to publications from recognised bodies, such as ‘Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing’ published by European Network of GMO Laboratories (ENGL) and the IUPAC ‘Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis’, for guidance on method validation.

(vi) DNA assessment: for analysis of foods containing several ingredients or having been processed, laboratories shall verify that the extraction and clean-up procedures used are capable of extracting good quality

amplifiable DNA and the resultant extracts are free from inhibiting substances. Procedures and methods used shall be designed so as to minimise the risk of false negative results due to the presence of inhibitors of nucleic acid amplification or restriction enzyme activity. Extraction method shall be validated for their ability to remove inhibiting substances.

(vii) Quality of the extracted DNA from all samples shall be assessed by well-established methods (e.g. gel based assessment and amplification of a 'housekeeping' gene). This could provide a means to assess whether the DNA has lost its integrity, and in such situations further testing would be inappropriate.

(viii) For extraction method that has not been shown to remove consistently the inhibitors, an inhibitor control shall be used. The inhibition can be estimated by the amplification of another target nucleic acid expected to be present in all samples or a known DNA spiked in test samples at known concentrations.

(ix) A laboratory may wish to modify an existing accredited test procedure such that the modified test procedure is still considered as covered by its scope of accreditation. In such case, the requirements as given in the Appendix apply.

II <u>For detection of gene doping by PCR</u>

(a) Test methods

(i) Test methods shall include information on the target regions of transgene to be tested as well as the binding location for each primer and probe so as to avoid incorrect interpretation of results.

(ii) When a screening test is used for gene doping detection, it shall be validated to demonstrate the limit of detection of individual transgene fragments claimed. Multiple PCR approach shall be employed by using multiple probes and specific primer sets targeting at at least two different regions of the transgene (e.g. two different exon-exon junctions) to provide the required specificity for claiming the full coverage of the target transgene, otherwise, only the result related to the detected region of the transgene can be reported.

(iii) For a screening test that targets at only one region of transgene by one primer-probe set, whenever technically feasible, the laboratory should develop a second PCR assay (e.g. another primer-probe set at different hybridisation sites within the same exon-exon junction) to minimize the risk of false negative results.

(iv) If a screening test is positive, confirmation of the detected region of the transgene shall be conducted. The laboratory shall have documented instruction for interpreting and reporting the presumptive adverse and atypical analytical findings.

(b) Method validation

(i) Laboratories shall determine the method performance characteristics including limits of detection, DNA extraction efficiency, nucleic acid purity and integrity, PCR specificity and sensitivity, amplification efficiency and linearity, precision, trueness and robustness, for in-house developed tests and tests based on modified standard methods, wherever applicable. Laboratories may refer to publications from recognised bodies for PCR based analysis and/or doping control testing such as ISO 20395 ‘Biotechnology – Requirements for evaluating the performance of quantification methods for nucleic acid target sequences – qPCR and dPCR’, ‘Gene Doping Detection based on Polymerase Chain Reaction (PCR)’ published by World Anti-Doping Agency and ‘The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments’, for guidance on method validation.

(ii) Procedures and methods used shall be designed so as to minimise the risk of false negative results owing to poor DNA extraction efficiency and/or the presence of inhibitory substances that inhibit the downstream nucleic acid amplification. The laboratory shall verify the extraction and clean-up procedures used are capable of extracting good quality amplifiable DNA and removing PCR inhibiting substances from resultant extracts.

(iii) Extraction method shall be validated for the absence of inhibitors and the integrity of extracted DNA from all samples suitable for further PCR testing by using extraction positive control. These can be

estimated by the amplification of another target nucleic acid expected to be present in all samples or a known DNA spiked in test samples at known concentrations by well-established methods (e.g. gel based assessment, spectrophotometry, fluorometry and/or amplification of a 'housekeeping' gene).

(iv) The limit of detection (LOD) is the minimum concentration of the target DNA in sample at which an amplicon is detected with a probability of at least 95%. In any case, the LOD shall not be higher than 2500 copies per mL.

(v) False negative rate shall be considered in the establishment of LOD. LOD shall be determined by a statistically valid approach at the required confidence level under the experimental conditions specified in the method.

(vi) PCR primers and probes designed to a target DNA sequence may bind to non-target sequence leading to non-specific amplification. Specificity of the method shall be demonstrated both theoretically or experimentally by: -

(1) in-silico specificity screen of oligonucleotide sequences (primer, probe) against the target amplicon sequence using suitable genome database (e.g. BLASTn);

(2) confirmation of the identity of amplicon by DNA sequencing;

(3) confirmation of the identity of amplicon by confirmation methods adopted in in-process quality control check in Cl. 7.7 (c)(vii); and

(4) evaluation of cross reactivity for exclusivity and inclusivity with non-target and target DNA sequences close to their claimed limits of detection during method optimization.

(vii) A laboratory may wish to modify an existing accredited test procedure such that the modified test procedure is still considered as covered by its scope of accreditation. In such case, the requirements as given in the Appendix apply.

7.3 Sampling

(a) Sampling from sample lot or site is not covered by this document. Customers taking their own samples shall be made aware of proper storage, sampling and transportation procedures.

(b) Laboratories shall document the minimum sample size particularly for raw materials and sub-sampling procedures for taking test portions from laboratory samples. Laboratories shall have measures to ensure that the test portion is representative of the laboratory sample, and the composition of the sample shall not be altered in a way that would affect the concentration of the target DNA being determined. Preparation of laboratory samples and test portions, if not specified in test standards, should be based on national or international standards specific to the products to be tested. Sample size shall be sufficiently large to provide statistically meaningful data at the limit of detection of the method. Customers should be informed if sample received is too small for meaningful analysis.

7.4 Handling of test or calibration items

(a) Laboratories shall examine and record the conditions and appearance of samples upon receipt. Items to be checked include nature and characteristics of sample, volume/amount of sample, conditions of sample container, characteristics of the sampling operation (sampling date and condition), etc. If there is insufficient sample or the sample is in poor condition due to physical deterioration and torn packaging or deficient labelling, laboratories shall either refuse the sample or carry out the tests with written instructions from the customers and shall indicate the conditions and possible effects on the test results on test reports.

(b) Samples pending testing shall be stored under suitable conditions to minimise any degradation. Storage conditions for samples and sample extracts shall be documented and shall fulfil the requirements of test standards.

(c) Frequently, it is necessary to split or transfer samples for testing of different properties. It is essential that procedures are available for preventing spread of contamination. Documented procedures and records on delivery of samples including special transportation such as refrigeration and exclusion of light, disposal and decontamination processes and unbroken chain of

identification of the sub-samples/samples shall be available.

7.5 Technical records

(No additional explanation)

7.6 Evaluation of measurement uncertainty

(a) It is recognised that the current state of knowledge regarding measurement uncertainty across the full range of PCR based analysis is limited. The measurement uncertainty evaluation methods given by reputable professional and standard writing bodies generally accepted within the testing discipline may be used. However, it is important that the measurement uncertainty obtained shall be in line with the definition given by JCGM 200 'International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM)' and includes all major components of uncertainty. Reference to the EURACHEM/CITAC Guide CG 4 'Quantifying Uncertainty in Analytical Measurement', ISO 5725-3 'Accuracy (trueness and precision) of measurement methods and results – Part 3: Intermediate measures of the precision of a standard measurement method', ISO 20395 'Biotechnology – Requirements for evaluating the performance of quantification methods for nucleic acid target sequences – qPCR and dPCR' and 'Guidance document on Measurement Uncertainty for GMO Testing Laboratories' published by Joint Research Centre of the European Commission may be useful.

7.7 Ensuring the validity of results

(a) Laboratories shall establish and implement quality control plans to ensure and demonstrate that the measurement process is in control and the test results generated are valid. The plans shall include types of quality control checks, their frequency and acceptance criteria, and actions to be taken when results have been out of specifications.

(b) It is common that quality control plans are stipulated in test standards. These plans and the specified criteria shall be followed strictly. If such plans are not given, Cl. 7.7 (c) to 7.7 (d) shall be followed where appropriate.

(c) In-process control check
The following controls shall be performed at a minimum frequency of once

every test run, unless otherwise specified: -

(i) Extraction negative control
The extraction buffer employed for DNA extraction. It shall be prepared from sterile water and shall be autoclaved prior to use.

(ii) Extraction positive control
A well-characterised sample with target nucleic acid expected to be present in all samples with defined extraction efficiency or a test sample spiked with known amount of target nucleic acid.

(iii) Negative PCR control
Sterile water and CRM(s) with statistically based 0% GM content for GMO testing or master mix without template DNA for gene doping detection if available. It shall be subjected to PCR-based analysis in exactly the same manner as samples.

(iv) Positive PCR control
Reference DNA or DNA extracted from a CRM or a known positive sample. It shall be incorporated to demonstrate the unique performance of the PCR assay.

(v) Detection limit control
A sample of known GM content (or template DNA content) or CRM meeting the limit of detection of the method. A method shall be established to determine the amount of DNA in the control extract and dilutions or adjustments shall be made to provide the correct amount of target DNA at its detection limit, if appropriate.

(vi) Replicate analyses
It is well known that duplicate extractions and PCR of the same sample can give qualitatively different results (one positive, one negative) where the positive result is not due to contamination. This situation is most likely to occur in cases where the test is working at concentrations close to the limit of detection and/or there is some degree of inhibition of PCR due to co-extractives from the sample. Therefore, duplicate samples (or duplicate matrix spikes at a concentration close to LOD) which go through the entire extraction and PRC process shall be analysed at a minimum frequency of once per batch of samples or type of matrix or twenty samples, whichever is more frequent, for qualitative,

semi-quantitative, and quantitative methods.

(vii) Number of primer sets / Primer-probe sets

It is normally expected that test results are based on the results of at least two different specific primer sets and/or primer-probe sets, each providing consistent results, for both conventional PCR and real time PCR. If only one primer set or primer-probe set is available, other means of confirming the identity of an amplicon shall be established and implemented. Typical confirmation methods include:

(1) for conventional PCR, restriction enzyme cutting producing fragments of the expected size, hybridization of the PCR products with specific DNA probes or DNA sequencing of the PCR products;

(2) for real time PCR, hybridization of the PCR product with another pair of primer-probe set at different hybridization sites, DNA sequencing or melting curve analysis of the PCR products.

(d) Normally, at least three standards (excluding zero concentration) shall be used to establish a linear calibration for an assay and to evaluate the PCR efficiency of an equipment. The standards used shall bracket the concentration range of the test samples for quantitative tests. The lowest standard shall be at a level close to the reporting limit for the quantitative test method and/or limit of detection (LOD) for the qualitative method. Criterion of the correlation coefficient of linear calibration graph shall be defined and implemented.

(e) Proficiency testing programme

(i) Proficiency testing programme shall be scheduled and implemented on a regular basis. The frequency of participation shall be at least once per year for each measurement technique, sample preparation method and DNA extraction method covered by the scope of accreditation. The proficiency testing programmes participated shall cover, as far as possible, all GM events (or transgenes) commonly encountered by the laboratory within 2 reassessment cycles. If proficiency testing programme is not available, inter-laboratory comparison may be conducted preferably with other accredited laboratories.

(ii) Laboratories shall document procedures for rectifying unsatisfactory

performance in proficiency testing programmes. If unsatisfactory results are obtained, laboratories shall promptly investigate the root cause, take actions to rectify the problem(s) and demonstrate that it can achieve satisfactory performance for the test/method in question after corrective actions have been implemented. All findings and actions taken in connection with unsatisfactory performance shall be recorded.

7.8 Reporting of results

(a) Test reports shall be clear and not be potentially misleading. Reports shall accurately describe both the primer sets / primer-probe sets performed and the results obtained. Detailed requirements for reporting of results are as follows:

(i) For detection and quantification of GMO by PCR: the specificity of the target sequence shall be reported, i.e. '35S promoter: detected', or 'Roundup Ready: not detected' or 'Bt-176: not detected' instead of a general statement 'does not contain GMO'. The latter wording would imply that primer sets covering all potential GM varieties had been run. Similarly, quantitative results shall be reported as 'x.x % of Roundup Ready Soybean' instead of 'x.x % GM material'.

(ii) For detection of gene doping by PCR: the specificity of the target sequence shall be reported. For reporting positive results, only the detected region of the transgene can be reported if only one primer-probe set was applied. E.g. 'hEPO (*X/Y* junction): detected' shall be reported instead of 'hEPO: detected'. For reporting negative results, general statement 'Transgene was not detected' shall not be made to imply that the primer-probe set covering all potential transgenes and its varieties had been run. Similarly, the negative results shall be reported as 'hEPO (*X/Y* junction): not detected' instead of 'Transgene: not detected'.

Note: For confidentiality reasons, other reporting formats as agreed with the customer may be used provided that the specificity principles as given in Cl. 7.8 (a)(ii) are fulfilled. In any case, the reporting of results shall not be misleading.

(b) When test results are below the reporting limits, an indication of the reporting limits shall be given in test reports.

(c) The sample preparation procedure shall be given if it is required for the proper interpretation of test results.

(d) In determining the decision rule to be applied when stating conformity with a legislation, specification or standard, international guideline, such as ILAC-G8 ‘Guidelines on Decision Rules and Statements of Conformity’, EURACHEM/CITAC Guide ‘Use of uncertainty information in compliance assessment’ or EUROLAB technical report ‘Decision rules applied to conformity assessment’ may be useful.

7.9 Complaints

(No additional explanation)

7.10 Nonconforming work

(No additional explanation)

7.11 Control of data and information management

(No additional explanation)

8 Management system requirements

(No additional explanation)

Appendix

Requirements for laboratories making modifications to an accredited test procedure for PCR based analysis for GMO and doping control testing

(a) The bounds within which the laboratory may modify its test procedures shall be clearly defined and approved by the HKAS Executive, and normally, a laboratory may:

I For detection and quantification of GMO by PCR

(i) include additional products using the same testing techniques for the test parameters for which the laboratory is already accredited;

(ii) include additional GM events using the same testing techniques for which the laboratory is already accredited;

(iii) modify the procedure of a particular method for a specific product for which the laboratory is accredited. This includes changes in DNA extraction methods (e.g. using a different brand of commercial kit) as well as DNA identification and quantification methods (e.g. changes in PCR annealing temperatures and/or time; changing a primer set specific to a particular taxon-specific gene).

II For detection of gene doping by PCR

(i) include additional transgenes using the same testing techniques for which the laboratory is already accredited;

(ii) modify the procedure of a particular method for a specific matrix for which the laboratory is accredited. This includes changes in DNA extraction methods (e.g. using a different brand of commercial kit) as well as DNA identification methods (e.g. changes in PCR annealing temperatures and/or time; changing a primer set and probes specific to a particular specific exon/exon junction).

(b) Any modifications shall not involve new analytical technique or principle not previously covered under the scope of accreditation of the laboratory. The laboratory shall validate that the modified procedures are fit for purpose and can meet the performance criteria required (or verify that the laboratory has the capability to conduct the modified procedures, in case such procedures have been fully validated by recognised bodies or through inter-laboratory comparison studies) in accordance with internationally acceptable guidelines before introducing such procedures in its scope of accreditation.

(c) The laboratory shall have demonstrated good system maturity and meet the accreditation criteria for Monitoring Plan B or C (as given in HKAS SC-04) for PCR based analysis.

(d) The laboratory shall demonstrate technical competence by obtaining satisfactory results in the latest two relevant proficiency test programmes or inter-laboratory comparisons within the previous two years using the accredited test procedures. It shall also demonstrate its technical competence to validate and verify test procedures in accordance with internationally acceptable guidelines and obtain no critical/significant nonconformity under Section 7.2 of ISO/IEC 17025:2017 in the last two assessments.

(e) The laboratory shall have gone through at least three applications for extension of scope of accreditation for the analytical technique/principle concerned.

(f) The laboratory shall have been accredited to perform the determination of at least 20% of the GM events (or 20% of the transgenes) anticipated to be covered by the modified test procedure for detection and quantification of GMO (or detection of gene doping) and shall cover representative and difficult test procedures, target DNA sequence and/or sample matrix.

(g) The staff who are responsible for the development and modification of test procedures shall have sufficient technical knowledge of the test and the technology used. They shall be able to judge the suitability of the test and the validity of the results obtained. They shall be approved signatory in the test concerned and have at least 1 year of experience in the sub-test area under consideration. HKAS Executive will specifically assess the competence of the staff who are authorised to undertake method development and modification during assessments, taking into consideration factors such as the staff's (i) formal education and training received; (ii) experience within the field; (iii) participation in research or development projects; (iv) participation in standardization committees; and (v) participation in scientific or authoritative committees.

(h) The process for developing, validating/verifying and authorising modified test procedures shall be controlled and documented. The process shall be reviewed at suitable intervals for adequacy and the related activities shall be monitored by incorporation into the laboratory's internal audit programme.

(i) The laboratory shall maintain a record system that can demonstrate how a test procedure was modified, validated/verified and accepted, the justification for any modification, and who was responsible for each key activity. The information recorded shall be sufficient to allow audits to clearly follow the events leading to the introduction of each modified test procedure.

(j) The laboratory shall demonstrate their technical competence to validate modified procedures in accordance with Cl. 7.2.2 of ISO/IEC 17025:2017 as well as Section 7 of this document.

(k) The laboratory shall ensure that modified procedures have been fully validated before they are introduced in its scope of accreditation. Modified procedures shall be reviewed regularly on the suitability of primers and probes. The laboratory shall ensure that primers and probes are able to consistently deliver the required performance for their intended applications.

(l) The laboratory shall implement sufficient quality control measures to ensure the validity of the results obtained from the modified procedures.

(m) If nonconforming testing work is identified in association with the use of any modified procedures, the work shall be handled in accordance with Section 7.10 of ISO/IEC 17025:2017, and in this connection, if any suspect inaccurate results are found reported to customers, the laboratory shall report the matter to HKAS Executive immediately.

(n) The laboratory shall notify HKAS Executive of any newly modified test procedures for incorporation into its scope of accreditation by submitting the modified procedures, the proposed scope and also the duly completed HKAS 009 form within 10 working days from the effective date of the modified procedures to HKAS Executive for review.

(o) The laboratory shall keep an updated scope of the tests the laboratory accredited to perform in the test area/sub-test area concerned, including any modified test procedures for PCR based analysis, the associated product/matrix types and GM events or target transgenes.

(p) The laboratory shall submit in full the validation/verification report with relevant raw data records, measurement uncertainties and other pertinent information as appropriate e.g. staff training records, for any newly modified test procedures since the last reassessment visit for review by HKAS Executive upon request.

(q) HKAS will closely monitor the performance of the laboratory, e.g. through unannounced or scheduled visits to the laboratory, matters arising as per Cl. (m) above, etc., and may amend or delete any items proposed by laboratory for inclusion into its scope of accreditation or terminate the practice of the laboratory under Cl. 7.2.2 I (b)(ix) and Cl. 7.2.2 II (b)(vii) in this document at its discretion.

Annex
(Informative)

Bibliography

Laboratory staff members responsible for PCR based analysis are strongly advised to consult the following references.

1. European Network of GMO Laboratories (ENGL) *Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing*

2. Pure Appl. Chem., Vol. 74 No. 5 IUPAC Technical Report *Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis*

3. ISO 20395 *Biotechnology – Requirements for evaluating the performance of quantification methods for nucleic acid target sequences – qPCR and dPCR*

4. World Anti-Doping Agency Laboratory Guidelines *Gene Doping Detection based on Polymerase Chain Reaction (PCR)*

5. Clinical Chemistry, 55:4 611-622 (2009) *The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments*

6. Joint Research Centre (JRC) of the European Commission Technical Report *Verification of analytical methods for GMO testing when implementing interlaboratory validated methods*

7. JCGM 200 *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM)*

8. EURACHEM/CITAC Guide CG 4 *Quantifying Uncertainty in Analytical Measurement*

9. ISO 5725-3 *Accuracy (trueness and precision) of measurement methods and results – Part 3: Intermediate measures of the precision of a standard measurement method*

10. Joint Research Centre (JRC) of the European Commission Scientific and Technical Report *Guidance document on Measurement Uncertainty for GMO Testing Laboratories*

11. ILAC-G8 *Guidelines on Decision Rules and Statements of Conformity*

12. EUROLAB Technical Report No. 1/2017 *Decision rules applied to conformity assessment*

13. ILAC-G7 *Accreditation Requirements and Operating Criteria for Horseracing Laboratories*

Remark: For dated references in the whole Annex, only the edition cited applies. For undated references cited, the latest edition (including any amendments) applies.