HKAS Accreditation Criteria on Molecular Testing of Food for Authenticity

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Introduction

- For the purpose of authentication, it is often necessary to identify the species from which food sample originates
- Species identification by DNA sequencing
- HKAS offers accreditation service on this type of tests
  - HOKLAS Supplementary Criteria No. 43
    - Interpret and amplify ISO/IEC 17025 requirements

Scope of Accreditation

- Foods that are composed of the whole or a part of the body of a single organism
- Foods either be unprocessed or have only been subjected to processing that does not introduce contaminating DNA from other species
- DNA sequencing technique

Limitations on Applicability

- There are foods for which DNA sequencing alone cannot confirm authenticity:
  - those not a body part of an organism or do not normally contain DNA of the species in concern
  - have undergone processing that would destroy DNA material or introduce contaminating DNA
- In these cases, DNA sequencing results can only be supportive evidence
- Other methodologies e.g. chemical analysis shall be employed for authentication

Requirements

1. Personnel
2. Accommodation and Environmental Conditions
3. Reagents and Equipment
4. Method Validation
5. Measurement Traceability
6. Sample Handling
7. Quality Assurance
8. Reporting

Personnel

- At least 1 member of technical management possesses adequate knowledge of and experience in molecular analytical methods
- Testing shall be performed by staff members who have undergone formal training in molecular analysis

Personnel

- HOKLAS approved signatories shall have as a minimum:
  - a bachelor's degree in molecular biology, biology, biochemistry or other relevant subjects; or membership of relevant professional bodies; or 10 years extensive experience in DNA sequencing
  - 3 years relevant testing experience
  - 6 months experience in DNA sequencing
  - adequate understanding of taxonomy and food science
- Candidates shall demonstrate to assessors their technical competence before signatory approval can be granted

Accommodation and Environmental conditions

- Procedures and precautions taken in avoiding cross-contamination
- Separate areas for
  - sample receipt
  - sample preparation and extraction
  - reagents preparation and dispensing of master mix
  - amplification, product detection
  - Post-PCR product purification and DNA sequencing
- Forward flow principle for sample handling
  - Unidirectional movement of DNA material

Reagents and Equipment

- Extraction kits, PCR kits, sequencing kits, enzymes, oligonucleotide primers, dye terminators and sequencing polymer shall be verified for performance before use
- *Taq* polymerase shall possess proofreading activity
- Thermocycler, sequencers and corresponding built-in spectroscopic components shall be verified regularly and well maintained
- Critical parameters that have effects on the validity of test results, e.g. temperature and time, shall be calibrated

Method Validation

- Laboratories shall preferably use national, regional, international standard methods
  - Verify own ability to achieve required performance

- Fully validated in-house methods

Method Validation

- Selection of target sequence(s)
  - Can unequivocally detect and identify specific organism/species
    - Discriminatory power against different species
    - Length of sequence (> 500 base pairs)
    - No. of sequences used in the method
- Extensive literature review before validation

Method Validation

- Selectivity against non-target closely related species (False positive rate)
- Minimum amount of DNA needed to yield positive results (False negative rate)
- Efficiency of extraction method
- Applicability in foods being processed
- Repeatability and Reproducibility
- Experimental design shall be fully documented

Measurement Traceability

- Reference sequence used for comparison shall be obtained from:
  - certified reference materials produced by reputable national institutes or authority
  - Reference materials authenticated by recognized national institutes or authority
  - Public genetic sequence database, given that the sequence is
    - submitted by two or more independent groups of researchers and the homology is 100%; or
    - submitted by a recognized laboratory that has published significant work on the target species

Measurement Traceability

- To infer homology the DNA sequence of the tested material shall match the relevant CRM or reference sequence at 98% or higher.

Sample Handling

- Samples submitted as individual items shall not be mixed for testing
- If not all items in the sample are tested, number of items selected for testing and total number of items received shall be recorded and reported

Quality Assurance

- Quality control plan shall include in each run:
  - Blanks (negative controls)
  - Extraction positive controls
  - Assessment of quality of extracted DNA
  - PCR/Sequencing positive controls
  - Repeat testing if no PCR products obtained
- Quality of chromatogram shall be evaluated
- Proficiency testing activities
  - At least once before accreditation granted
  - At least once per year per type of test

Reporting of Results

- For authentication:
  - "The sample is found to be *Species X* in origin based on the results of DNA sequencing"
  - "The species origin of the sample cannot be ascertained by the results of DNA sequencing"

Reporting of results

- Where DNA sequence analysis alone is not sufficient for authentication purpose:
  - "The sample was found to contain DNA from *Species X* based on the results of DNA sequencing"
- If conclusion cannot be drawn due to insufficient amount or poor quality of DNA or other technical reasons, it shall be stated clearly in the report

For more information:

HOKLAS Supplementary Criteria No. 43 – Species Identification by DNA Sequencing for Authentication Purpose

www.hkas.gov.hk