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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

OECD/UPOV/ISTA JOINT WORKSHOP ON MOLECULAR TECHNIQUES

Seoul, Republic of Korea, November 12, 2014

HORIZONTAL METHODS FOR MOLECULAR BIOMARKER ANALYSIS SC16

Document prepared by the International Organization for Standardization (ISO)

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation “Horizontal Methods for Molecular Biomarker Analysis SC16” to be made at the OECD/UPOV/ISTA Joint Workshop on Molecular Techniques.

[Annex follows]

ANNEX

Horizontal Methods for Molecular Biomarker Analysis at the International Organization for Standardization (ISO)

Subcommittee (SC) 16

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Subcommittee (SC) 16 Scope*

Develops Standards for biomolecular testing methods applied to

- Foods
- Feeds
- Seeds
- Other propagules of food and feed crops

Includes methods that analyze

- Nucleic acids [e.g., polymerase chain reaction (PCR), genotypic analysis and sequencing],
- Proteins [e.g. enzyme linked immunosorbent assay (ELISA)],
- Other suitable methods.
- Variety identification and detection of plant pathogens.

*The scope does not include food microbiological methods.

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SC 16 Administrative Structure

ISO Technical Committee (TC) 34/SC 16

- hosted by the American National Standards Institute (ANSI-USA)

The SC 16 secretariat and the US Technical Advisory Group

- administered by the American Oil Chemists' Society (AOCS)

  - Secretary: Dr. Richard Cantrill, USA, Technical Director, AOCS. (richard.cantrill@aocs.org)
  - ISO-Central Secretariat Technical Project Manager: Marie Noelle Bourquin
  - Committee Chair: Dr. Michael Sussman, Senior Research Scientist, US Department of Agriculture, Washington, DC, USA (michael.sussman@ams.usda.gov)
  - ISO US Technical Advisory Group Member, Expert and ISO Presenter: Dr. Paul Zankowski, Commissioner-US Plant Variety Protection Office, US Department of Agriculture, Washington, DC, USA (paul.zankowski@ams.usda.gov)

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SC 16 International Structure

Participating Countries (23)		
Argentina (IRAM)	Germany (DIN)	Russian Federation (GOSTR)
Austria (ASI)	India (BIS)	Slovenia (SIST)
Canada (SCC)	Iran, Islamic Republic of(ISIRI)	Sweden (SIS)
Chile (INN)	Ireland (NSAI)	Switzerland (SNV)
China (SAC)	Japan (JISC)	Thailand (TISI)
Denmark (DS)	Korea Rep. (KATS)	United Kingdom (BSI)
Egypt (EOS)	Namibia (NSI)	United States (ANSI)
France (AFNOR)	Netherlands (NEN)	
Observing Countries (10)		
Belgium (NBN)	Italy (UNI)	Serbia (ISS)
Croatia (HZN)	Poland (PKN)	Slovakia (SUTN)
Cyprus (CYS)	Romania (ASRO)	Spain (AENOR
Czech Republic (UNMZ)		

National Standards Body abbreviations in parentheses following each country/organization

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SC 16 Formal Liaisons

- ISO/TC 34/SC 9 (Microbiology)
- ISO/TC 69 (Applications of statistical methods)
- ISO/TC 69/SC 6 (Measurement methods and results)
- ISO/TC 93 (Starch)
- ISO TC 276 (Biotechnology)
- TC 212 (Clinical laboratory testing and *in vitro* diagnostic test systems):proposed
- American Association of Cereal Chemists International
- American Oil Chemists' Society
- European Communities
- European Environmental Citizens Organization for Standardization
- European and Mediterranean Plant Protection Organization
- International Plant Protection Convention
- The Functional Genomics Data Society

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Importance of standards from SC 16

- Provides an internationally harmonized platform for evaluating crop, food, and food product origin/identity/quality.
- Tools of molecular biology can
  - differentiate between crop varieties
  - identify food and food products based on differences and similarities in molecular structure.

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Importance of standards from SC 16

- The possibility for determining the nature of a specific crop, food, or food product
  - adds value
  - provides assurance for label claims and suitability for consumption.
- Standards for molecular identification and detection provide methodologies that
  - enable internationally transparent export and import of crops
  - enhances trade by reducing or eliminating technical barriers.

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SC 16's Role in International Commerce

- A diverse variety of seeds and plant products move through international markets, e.g. local and imported, specific varietals, genetically engineered (GE) and non-GE foods, and identity preserved and commodity crops.
- In this competitive and rapidly changing economic environment efficient and effective fair trade technologies for trait identification, varietal identity preservation and plant pathogen detection must become uniform, faster, and less expensive.
- SC 16 strives for an inclusive working environment, ensuring that opportunities are available for all nations to participate.

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ISO Deliverables

- Technical work in ISO is performed by nearly 300 technical committees covering many industrial fields.
- The primary assignment of a technical committee or subcommittee is the development and maintenance of **International Standards**.
- Other ISO deliverables include:
  - **Technical Specifications** - subject areas which are under development
  - **Publicly Available Specifications** - usually a preexisting or intermediate specification distributed before an International Standard
  - **Technical reports** - a collection of data, representing a survey or the state of the art in a field

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Project stages and associated documents for ISO standard development*

Project stage		Associated document
1) **Preliminary stage**	→	Preliminary work item (PWI)
2) **Proposal stage**	→	New work item proposal (NP)
3) **Preparatory stage**	→	Working draft(s) (WD)
4) **Committee stage**	→	Committee draft(s) (CD)
5) **Enquiry stage**	→	Enquiry draft (ISO/DIS IEC/CDV)
6) **Approval stage**	→	Final draft International Standard (FDIS)
7) **Publication stage**	→	International Standard (ISO)**

** At each stage – a vote among participating nations is held to determine consensus agreement*

Accelerated standards development track — 24 months to publication
Default standards development track — 36 months to publication
Enlarged standards development track — 48 months to publication

**International Standards published by ISO are subject to systematic review every 5 years to determine whether it should be confirmed, revised/amended, converted to another form of deliverable, or withdrawn

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Published and Developing Standards for Varietal Identification

ISO 13495:2013* Foodstuffs -- Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid
ISO 16578:2013* Molecular biomarker analysis -- General definitions and requirements for microarray detection of specific nucleic acid
ISO/WD TR 17622 Molecular biomarker analysis -- SSR analysis of sunflower
ISO/DTR 17623 Molecular biomarker analysis -- SSR analysis of maize
ISO/AWI 19048 Molecular biomarker analysis -- Multiplexed SSR analysis for varietal identification of basmati rice
* Published

ISO 13495:2013

Foodstuffs -- Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid

http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=53822

Available in English and French Languages

ISO 13495:2013

- Specifies molecular tools for generating molecular profiles of varieties of plant species
  - enabling varietal identification
  - confirmation of identity in relation to one or more references.
- Applicable to various matrices, seeds, leaves, roots, industrial products composed of only one variety.
- Matrices presented in the form of mixtures of varieties (such as purees, compotes, flours) are excluded from the scope of ISO 13495:2013.
- Does not deal with genetic purity.

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ISO 13495:2013

- Supports decision-making and validation on the protocols that will be used to produce high-quality molecular data for varietal identification.
- This varietal identification testing requires high-quality markers, which are able to provide reproducible data using a range of equipment, chemistries and reagents.
- This standard only goes on to describe specific amplification methods.

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ISO 13495:2013

- Aims is to ensure that
  - methods of analysis are compatible with customer requests
  - lists the different steps towards method validation
  - defines acceptance criteria

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ISO 13495:2013

- Guarantees that the general principles employed in performing these analyses will be the same across all laboratories with regards to
  - reference material
  - sample size/laboratory sample/test portion
  - homogenization/grinding/extraction
  - results analysis and interpretation
  - certificate of analysis
- Plays a role in standardizing the results obtained by different laboratories.

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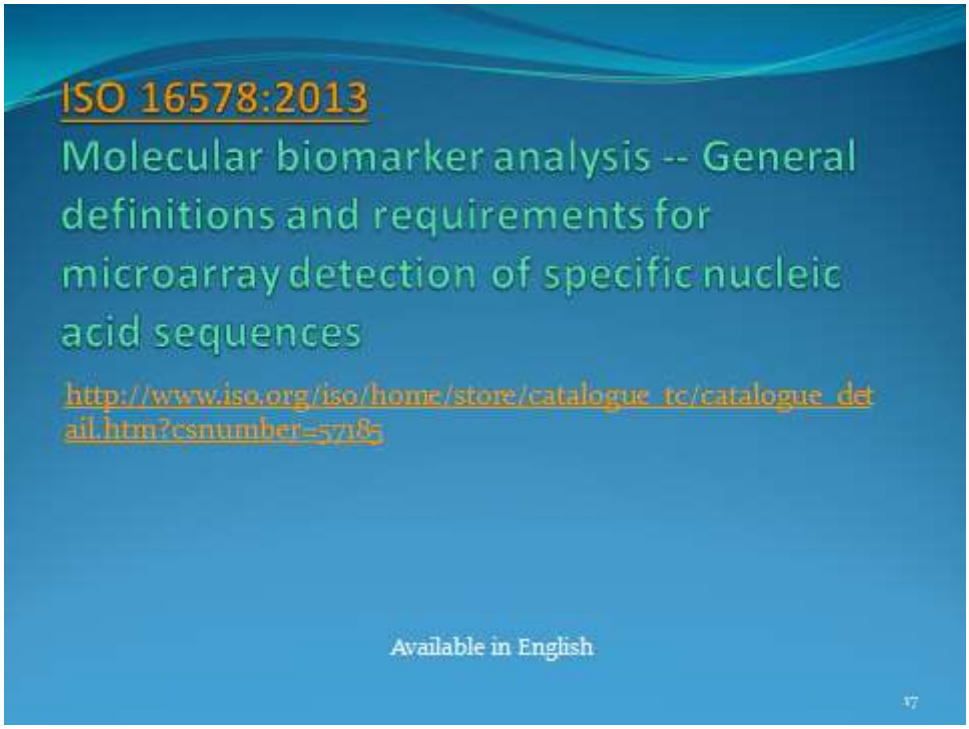
ISO 16578:2013
Molecular biomarker analysis -- General definitions and requirements for microarray detection of specific nucleic acid sequences
http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=57185
Available in English
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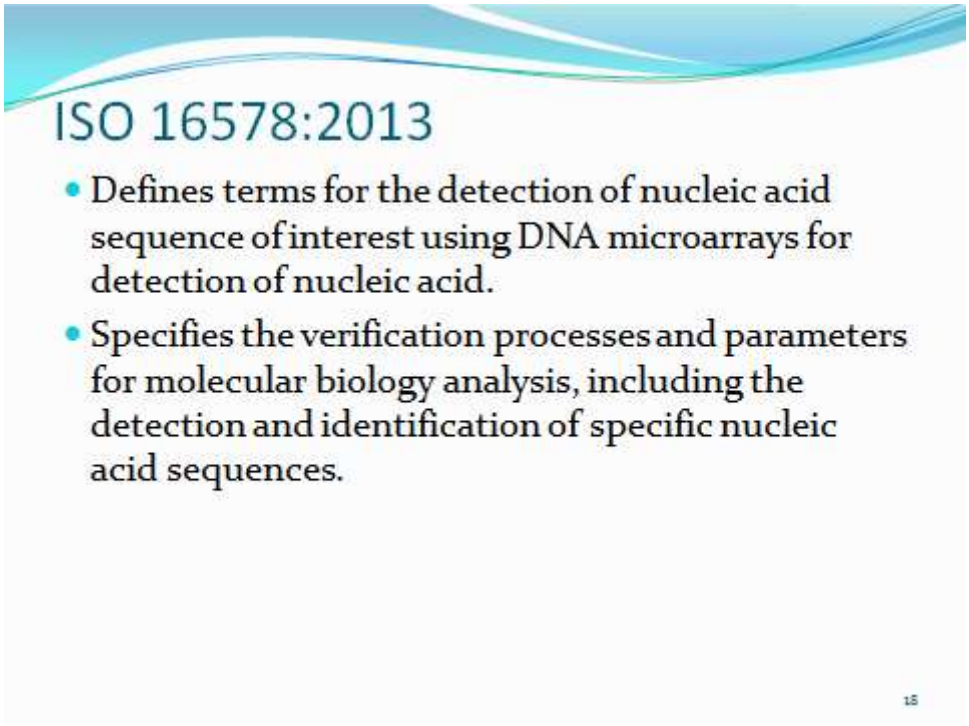
ISO 16578:2013
• Defines terms for the detection of nucleic acid sequence of interest using DNA microarrays for detection of nucleic acid.
• Specifies the verification processes and parameters for molecular biology analysis, including the detection and identification of specific nucleic acid sequences.
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ISO 16578:2013

- Provides recommendations and protocol for
  - microarray design and manufacture
  - validation of hybridization specificity
  - inter-laboratory validation of qualitative methods
  - determination of limits of detection for a microarray
  - determination of range of reliable signals
  - criteria to assessing technical performance of the microarray platform

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ISO 16578:2013

- DNA microarray
  - a technique capable of simultaneous detection of multiple nucleic acid sequences
  - particularly suitable for identifying nucleic acid sequences of interest and for measuring gene expression levels.
- Microarray and its derived technology have been developed for use in the field of food analysis including
  - genetically engineered organism (GEO) analysis
  - biomarker identification

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ISO 16578:2013

- Although the standardized parameters required for DNA microarray-based methods have been under consideration (such as MAQC and MIAME), it is necessary to generate minimum requirements for the interpretation of the results.
- The aim of this International Standard is to provide guidance and requirements for the detection of nucleic acid sequences of interest by microarrays. This information concerns
  - the establishment of validation approaches for methods based on DNA microarray, and
  - the definition of general principles employed in carrying out these laboratory analyses

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ISO 16578:2013

- Microarray technology is evolved from Southern blotting
- Core principle is hybridization between two DNA strands, by the property of complementarities of nucleic acid sequences.
- A DNA microarray is a collection of microscopic DNA spots attached to a solid substrate or coded beads.

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ISO 16578:2013

- The development of a microarray assay generally needs to
  - design the probe DNAs
  - arrange the probe DNAs onto a solid substrate
  - label the target nucleic acid sequences
  - hybridize the targets with the probe DNAs
  - elaborate an appropriate detection system.
- According to the target labelling techniques used, the hybridization can be detected by electrical, colorimetric, and/or fluorescence signals

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ISO 16578:2013

- At the time of publication of this standard, best practices and standards for data representation and minimum information have been developed for comparability and reproducibility of microarray data.
  - Only a few published works have yet focused on the reliability and comparability of any given microarray platforms, and a single-laboratory validation would most likely not suffice in this case.
  - An inter-laboratory validation method should be adopted, according to specific international guidelines.

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[End of annex and of document]