PCR validation approaches in different countries - comparability of detection methods

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Why testing for Biotech traits?

- Compliance with the law
  - Thresholds
  - Labeling
- IP-systems, grain channeling programs
  - Proof integrity
  - Maintain integrity
- Seed production
  - Quality Assurance
  - Inventory Management

- Give concerned consumer a choice

Elements of Analysis

- Sampling
  - Driver: representative sample
  - Established guidelines (ISO, FOSFA, GAFTA, etc.)
- Extraction
  - Driver: efficient extraction and purification of analyte
  - How to assess quality and quantity of analyte?
- Determination
  - Detecting trait (e.g, herbicide-tolerance)
  - Detecting proteins
  - Detecting DNA
- Calculation
  - How to relate to labeling provisions?

Test methods

DNA → Protein → RR-tolerance

PCR (polymerase chain reaction) based on DNA analysis (the genes) ➔ all

Protein-based techniques (ELISA, lateral-flow devices) ➔ most of current AgBiotech traits

Based on phenotype (Bioassay) ➔ herbicide tolerances

- Existing technology was utilized for threshold testing only (0.9%-5%)

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Method validation

- Validation (e.g., ISO5725, ISO/IUPAC/AOAC harmonized protocol) and standardization on an international scale are a must (e.g., CODEX, ISO)
  - Sensitivity
  - Specificity
  - Reproducibility, Repeatability
  - Ruggedness
  - Etc
- ➔ Demonstrated fitness-for-purpose
- Reference materials are needed
  - We have cooperated with NMIs (IRMM in Europe) for the production of corn and soy reference materials for food and feed testing

Interpretation of test results

- How to relate test results to labeling provisions?
- Test results
  - Protein in absolute quantities
  - DNA in absolute or relative quantities
  - How to compare protein test results (seed/grain) with DNA-based results (food/feed)?
  - How to compare test units of % seed (seed/grain) with test units of % DNA or weight-%?
- Labeling provisions
  - Labeling provisions in %
  - No threshold (China)
  - No units explicitly given (Europe 0.9%)
    prevailing assumption weight-%
    under dispute
  - Weight-% (Australia)

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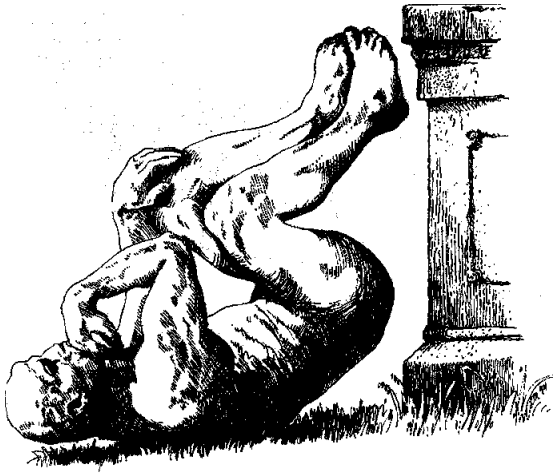
More challenges



- Biological factors:
  - Protein:
    - Expression level
    - Degradation
  - DNA
    - Zygosity/Hybrid status
    - (Selective) Degradation
      (Specific) Inhibition
    - Tissue specific genetics (corn)
- Measurement uncertainty
  - Exponential systems
  - Measurement unit (weight %, %-DNA)

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How to translate in the supply chain of food

- R&D
- Seed

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- Farmer → grain
- Commodity markets
- Food/feed processor

- Retailer (final food, final feed)
- Consumer

Regulator

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The European Model

- Biotech company:
  - Real-time PCR, event specific
  - in-house validation data (2 or more laboratories), very stringent criteria
  - Reference materials (ground seeds), food/feed samples
- Community Reference Laboratories
  - Multi-center validation study
- Modular validation favored, but no data for validity have been made available.

The Japanese Approach

- Regulators develop detection method Official methods do not need to be event-specific, most are construct-specific
- Methods need to be robust and fully validated

- Reference Materials: plasmid
- Close collaboration with Korea and other countries in SEA

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Seed → Grain → Food

- Seed → Grain: Genetics: single kernel or bulk materials

100% GMO | Bt0 X Bt0 | **Seed** (100% GM kernel)

100% GMO | BtBt, Bt0, Bt0, 00 | **Grain** (75% GM kernel)

GMO?

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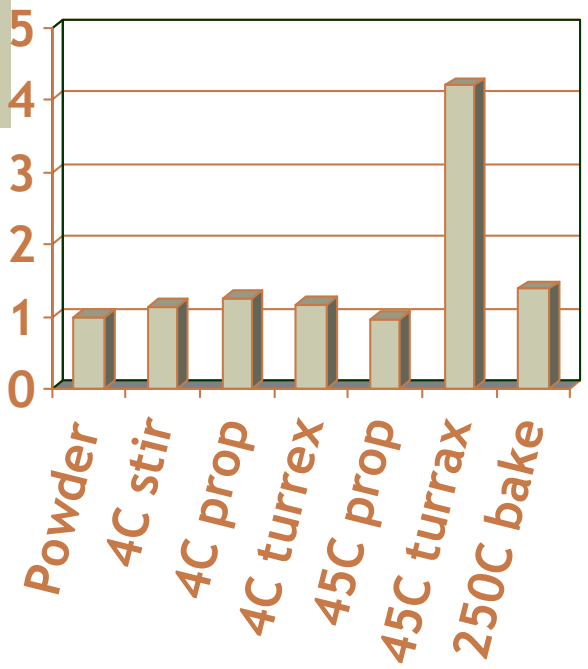
Seed → Grain → Food

- **Grain → Food: Genetics**

- Endosperm: triploid (2 maternal, 1 paternal) **starch**
- Embryo: diploid (1 maternal, 1 paternal) **oil, feed**

- **Grain → Food: Processing**



(H. Schimmel, Los Angeles, 2002)

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Testing throughout the supply chain

Regulation

Test most reliable

Seed | Grain | Ingredient | Final Food

Processing, complexity

Integrity

Applicability of testing

Protein test (preferred)
DNA tests

DNA tests

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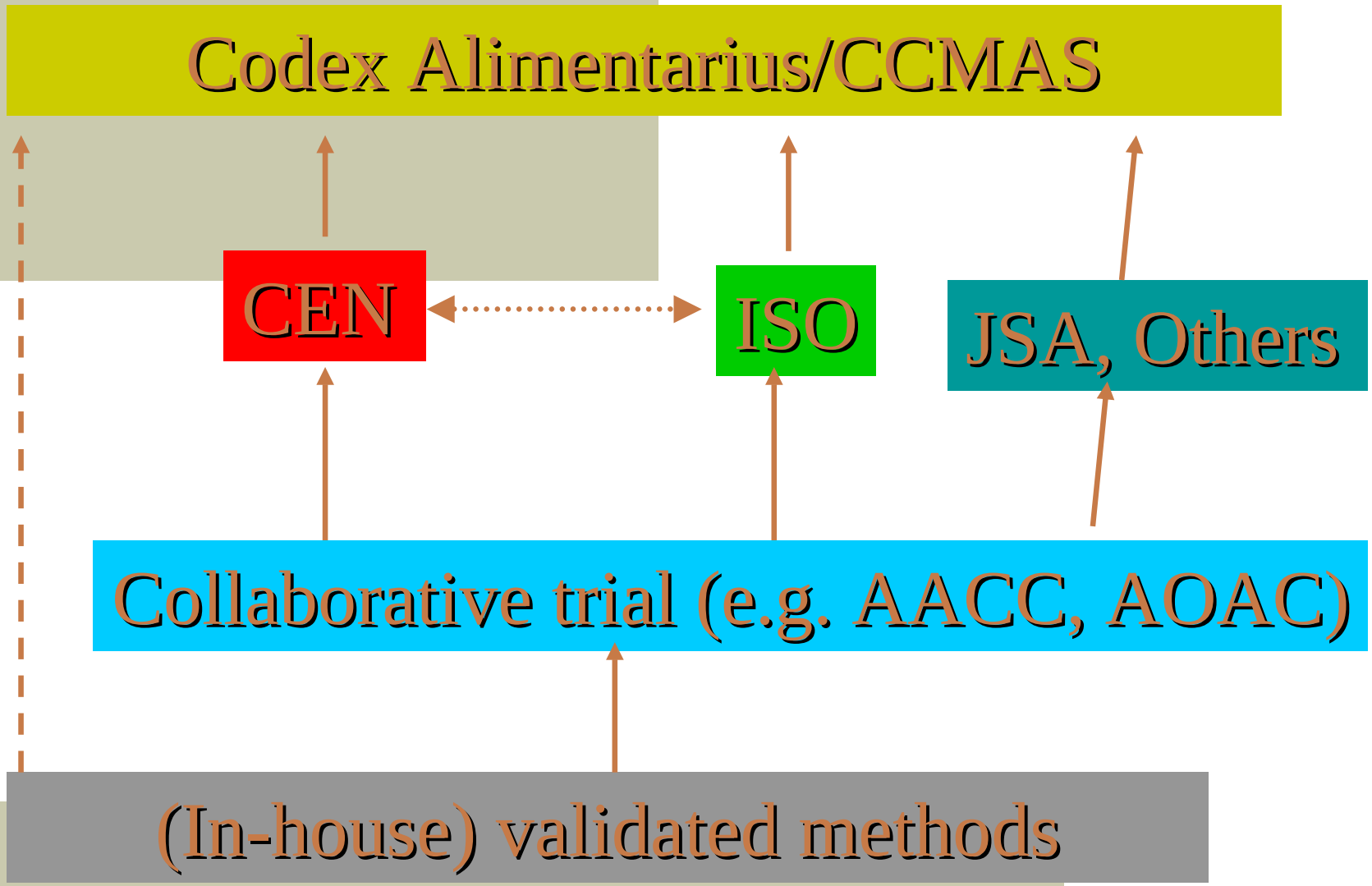
Need for standardization

- International, **global,** harmonization of validated detection methods is needed
- Validation shall encompass **all** analytical procedures (incl. extraction)
- Validation shall occur according to **international guidelines** (e.g, Harmonized protocol)
- Laboratories should work in compliance with a **Quality system** (e.g., GLP, ISO 17025)
- **Goal**: global comparability of test results

Coordination of Standardization Initiatives (food and feed)



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Reference materials

- Reference materials
  - Build consistent basis for global harmonization
  - Needed for calibration
  - Need for quality control
  - Etc
- Reference materials need to harmonized on an international level
  - Duplication is redundant and should be avoided
- Bureau of Weight and Measurements (BIPM) has set up a memorandum of mutual recognition amongst different Institutes
  - e.g., IRMM, NIST, NRC
  - We are working with these groups to ensure highest degree of harmonization

Summary

- **Testing of raw agricultural commodities gives most reliable results**
- **What is needed:**
  - All test options need to be evaluated for optimal choice (costs, time of analysis, etc); guidance on test units and their conversion
  - Bioassays, protein-based test are very reliable for seed/grain testing
  - DNA-based tests are suitable for food and grain testing
    - Expensive and sophisticated
    - Only expert labs give reliable results
- **Standardization**
  - Is on-going and needed on an international level
  - It is important to validate the whole analytical process/method
- **Reference Materials**: are an essential asset to ensure consistency and compatibility of analytical results