Workshop and Training Program on Sampling and Detection Methods Applied to Transgenic Crops

November 17 – 19, 2011, NIN, Hyderabad, India

ILSI International Food Biotechnology Committee

Test Method Performance Criteria and Validation

Anne Bridges. AACC Intl

Validation and Proficiency Testing

- Validation and proficiency testing ensure that a measurement made in one country/laboratory is the same as a measurement made on the same shipment in another country/laboratory
- Vital for trade in goods
  - *Methods* are tested at multiple laboratories.
  - *Laboratories* are tested using samples which are sent to them.

What is Method Validation?

"The process of establishing the performance characteristics and limitations of a method and the identification of influences which may change these characteristics and to what extent.

EURACHEM Guide

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Method development

Optimized method in a single lab

Pre-validation

Indication, if method works in different labs

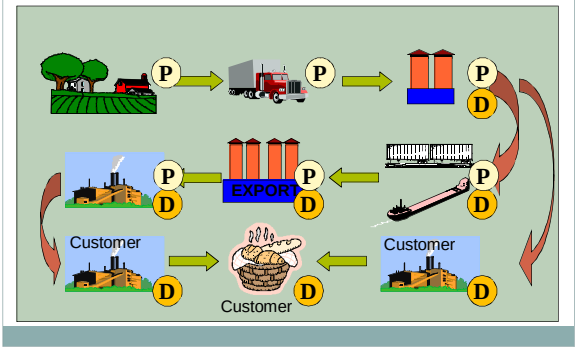
Collaborative study

Preferred approach

What criteria must be met by a method?

- Able to identify and quantify the analyte under investigation (Protein or DNA)
- Uses validated procedures
  - reliable, sensitive, practical, easy-to-use, inexpensive
- Easy to perform
  - Technically simple, Minimal steps
  - Difficult to perform incorrectly
- "Fit For Purpose"

Goal of Validating Methods



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International Guidelines Exist

ISO 5725 – International Standard for Validating a Method

Accuracy (trueness and precision)

- General principles and definitions
- Basic method for the determination of repeatability and reproducibility of a standard measurement method
- **Intermediate measures of the precision of a standard measurement method.**
- **Basic methods for the determination of the trueness of a standard measurement method**
- **Alternative methods for the determination of the precision of a standard measurement method**
- **Use in practice of accuracy values**
- **Guidelines for the evaluation of conformity with specified requirements**

- ***Guidelines also from AOAC, IUPAC, AACC Intl***

International Guidelines Exist

Appendix D: Guidelines for Collaborative Study. Procedures To Validate Characteristics of a Method of Analysis

AOAC OFFICIAL METHODS OF ANALYSIS (2002)

Minimum number of materials — Five
Minimum number of laboratories — Eight reporting valid data for each material
Minimum number of replicates — One, if within-laboratory and repeatability parameters are not desired; 2, if these parameters are required.

AACC International Method 11-30.01

- Quantification of MON 810 Corn in Corn Flour by Real-Time Polymerase Chain Reaction
- It is annexed to standard ISO 21570:2005
- Community Reference Laboratory for GM Food and Feed. 2006. CRL assessment on the validation of an event specific method for the relative quantitation of maize line MON 810 DNA using real-time PCR as carried out by Federal Institute for Risk Assessment (BfR).
- Validation Report Maize MON 810.

http: //gmo-crl.jrc.ec.europa.eu/summaries/Mon810 validation_report .pdf

General Approach Collaborative Trial

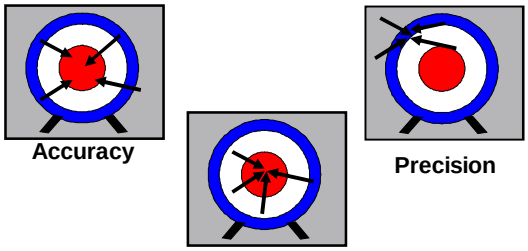
- Collaborative committee formed
- Method is pre-trialed at small number of labs
- Samples prepared, distributed to labs with exact method directions, study performed and data reported
- Results are evaluated and if no problems then a full size collab. trial is conducted
- Results are collected, appropriate statistics are performed
- Performance characteristics are calculated
- Method is written and approved

Which criteria must be tested during method validation

- **Accuracy**
- **Precision**
- **Specificity**
- **Sensitivity**
- **Repeatability**
- **Reproducibility**
- **Applicability**

Accuracy and Precision

Accurate and Precise Detection Methods



Accuracy and Precision

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Specificity and Sensitivity

Only the analyte under investigation should give a positive signal!

- testing different species/varieties, and
- testing different GMs used for food purposes

Sufficient sensitivity should be reached by the method; at least below a specified threshold

- Testing different concentration of the analyte (preferable CRM and/or matrix to be tested)
- Setting criteria to which the method must perform (related to RSD or threshold)

Repeatability of a Method

- **Variability within a lab**
- **Testing the precision under intra-lab conditions**
- **Same method**

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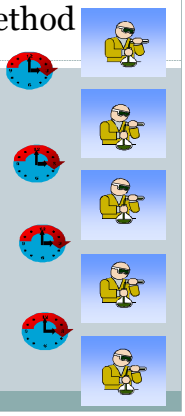

Reproducibility of a Method

- **Testing the precision under reproducible conditions**
- **Same method**
- **Different laboratories**
- **Preferably on international level rather than on a national level**

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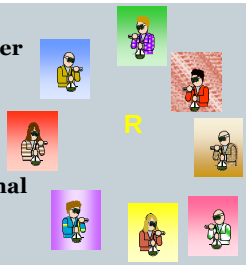

CODEX puts emphasis on "fully-validated" methods through collaborative trials

Precision Data – Method 11-30.01

Nominal	<0.02 %	0.10%	0.50%	1%	2%	5%
Labs reporting	11	14	14	14	14	14
Labs outlying	1	1	0	2	0	0
Labs used	10	13	14	12	14	14
Mean (%)	0.028	0.1023	0.4613	0.8327	1.7814	4.5154
s(*r*) (%)	0.007	0.03641	0.9606	0.13744	0.28385	1.29374
s(*R*) (%)	0.023	0.04646	0.20068	0.26534	0.56609	1.65451
RSD(*r*) (%rel)	26.27	35.60	20.82	16.51	15.93	28.65
RSD(*R*) (%rel)	83.03	45.43	43.5	31.86	31.78	36.64

s(*r*) = repeatability standard deviation, s(*R*) = reproducibility standard deviation, RSD = relative standard deviation, *r* = repeatability, and *R* = reproducibility.

Method Performance (PCR)

Type	Single lab and single technician Reference materials (DNA solutions) Regimented protocols and expectations	RSD (R)
Intra-laboratory	Collaborative Trial (Ring) Reference materials provided (DNA or flour) Reagents provided All major components controlled	10 – 20% [1]
Inter-laboratory	Proficiency Studies Ground material provided (may or may not be reference material) Reagents recommended but not provided Protocols provided but lab has latitude to make decisions	17 – 30% [2]
Inter-laboratory	Single lab and single technician Reference materials (DNA solutions) Regimented protocols and expectations	30 – 60% [3]

Brodmann *et al.* 2002, [2] CX/MAS 02/8, [3] AACC

The EU Community Reference Laboratory for GM Food and Feed

Role in approval of methods that are "fit for the purpose of regulatory compliance"

Key role in the EU for disputes and in response to crises

Method assessment is done according to ENGL-CRL set performance criteria.

Validated methods and validation reports are published at

http://gmo-crl.jrc.ec.europa.eu/

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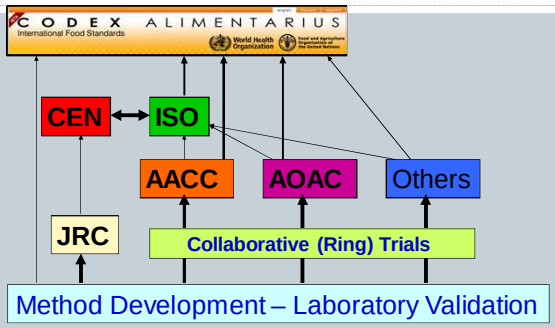
Validation - Methods

- Validation of **methods** is done through officially recognized and experienced bodies
  - Governmental bodies in collaboration with enforcement laboratories
    - **EU: CRL in collaboration with ENGL**
  - Global: AACC Intl, AOAC Intl, AOCS in many countries and >8 laboratories (usually ~ 20).
- Validated methods can be officially recognized by standardization bodies
- These methods can become the international reference

Coordination of Standardization Initiatives



Application of the Method

- **In what matrices does the method perform**
  - Reference material, raw commodity, finished food
- **Range of % GM the method has been validated over**
  - Was the threshold (or limit) required in analysis covered in the method validation study

Codex methods must be validated to show they are fit-for-purpose

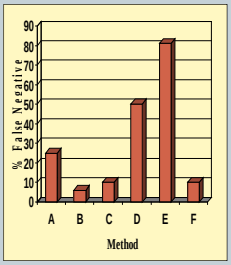
Are Methods Fit for Purpose?

- Usually developed with reference materials
- Validated for corn flour/soybean meal
- Applied to seed, grain, processed products, foods.
- Have they been validated to be "fit for purpose"?

Extraction Chemistry Matters

(% False Negative for 0.1% GM)

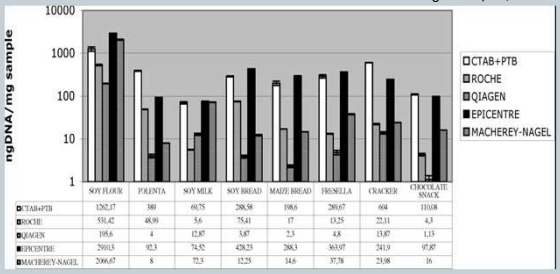
- Solid matrix precipitation
  - Method A 25%
  - Method B 6%
  - Method C 10%
- Selective precipitation
  - Method D 50%
  - Method E 81%
- CTAB/NaCl
  - Method F 10%



Holden et.al 2003

Extraction Chemistry Matters

G. Di Bernardo *et al* Biotechnol. Prog. **2007,** 23, 297-301



	SOY FLOUR	POLENTA	SOY MILK	SOY BREAD	MAIZE BREAD	FRISELLA	CRACKER	CHOCOLATE SNACK
CTAB+PTB	1262,17	380	60,75	288,58	108,6	280,67	604	1310,8
ROCHE	531,42	48,99	5,6	75,41	17	13,25	22,11	4,3
QIAGEN	105,6	4	12,87	3,87	2,3	4,8	13,87	1,13
EPICENTRE	2503,5	92,3	74,52	428,23	288,3	363,97	241,9	97,87
MACHEREY-NAGEL	2066,67	8	72,3	12,25	14,6	37,78	23,98	16

Figure 1. Amount (and standard deviations) of DNA extracted from maize and soybean foodstuffs with the five different extraction methods.

Summary

- Testing needs to be effective, consistent, and predictable along the supply chain to satisfy commercial requirements of trade
- Test method developers need to validate methods using internationally acceptable approaches
- They must demonstrate the methods are fit for purpose and transferable to practical testing environments
- Need to adequately measure all of the uncertainty associated with the "total method"

Thank you

Questions?