



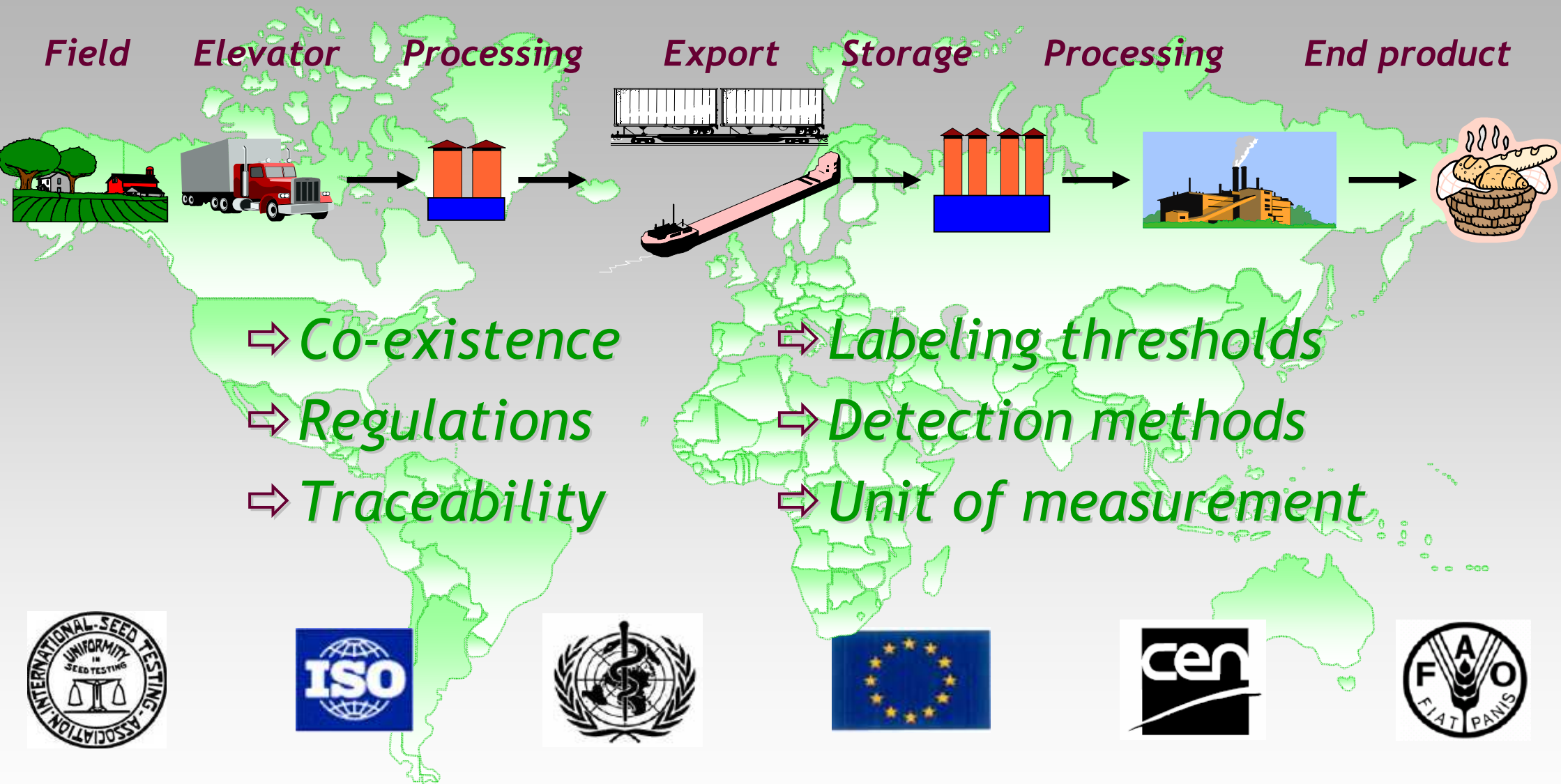
Bayer CropScience

BioScience

***Current Status of
Analytical GM Seed & Grain Detection***

Erik van der Biezen

Consistent GM testing along the food/feed chain



Analytical GM Detection & Thresholds

Authorized GM crops have undergone scientific safety evaluation & risk assessment

GM cultivation is not a safety issue but a question of consumers choice & economic impact

Co-existence allows conventional crops with presence of GM seeds below a threshold

⇒ *Therefore, legislation for GM seed thresholds is required*

⇒ *For co-existence procedural legislation is required*

Thresholds are key to

⇒ *Workable co-existence management*

⇒ *Regulatory approvals*

⇒ *Labeling*

⇒ *Consumer acceptance*

⇒ *Development & validation of detection methods*

Setting Standards



No testing possible without setting standards

- ⇒ *Threshold* → Maximum acceptable level of GM seed in a lot
- ⇒ *Level of confidence* → How sure you are of the result

“Zero GM” can only be determined by testing every kernel

- ⇒ *Every kernel tested = Every kernel destroyed*

“Not Detected” does not mean “zero GM”

- ⇒ *It means that it is below a certain threshold with a specified level of confidence*
- ⇒ *E.g. No GM seed out of 3,000 seeds → Lot impurity below 0.1% at 95% confidence*

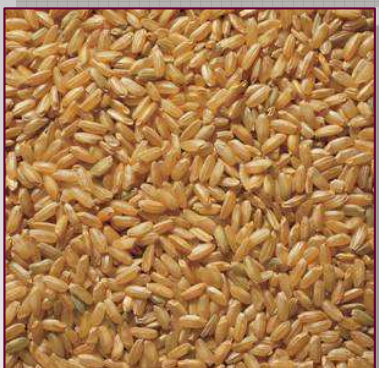
Conflicting test results?

- ⇒ *At low GM levels, one may get different results from testing more than once*
- ⇒ *Possible for 1 test to find “not detected” while a 2nd test may be positive*

Thresholds & Limit of Detection (LOD)

The lower the threshold, the more seed/grain must be sampled & analyzed to achieve it

- ⇒ Low thresholds are costly*
- ⇒ Thresholds determine the total number of seed / grain to be analyzed*
- ⇒ Number of tested samples is determined by the Limit of Detection (LOD)*



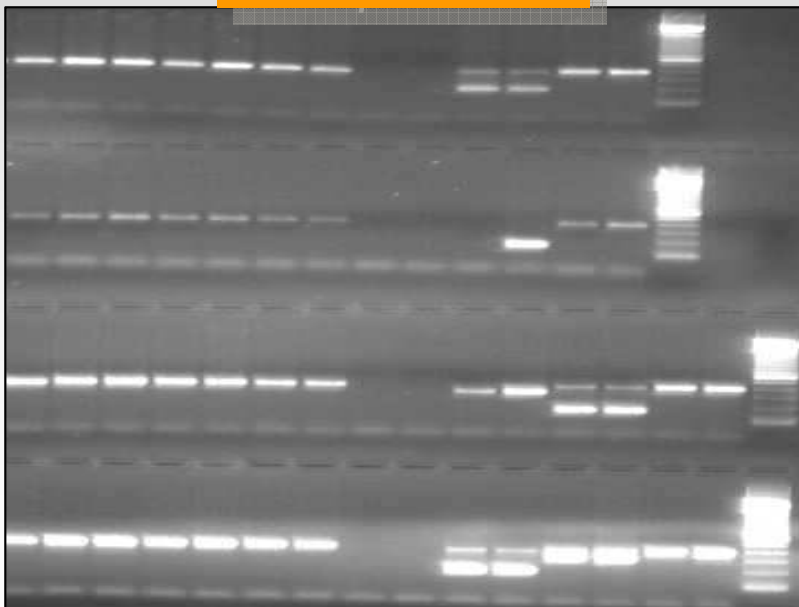
LOD = lowest quantity GM seed that can be detected with high level of confidence

<u>Threshold</u>	<u>Number of seeds/grains to be tested (95% confidence)</u>	
1%	→	300 seeds
0.1%	→	3,000 seeds (3 x 1,000)
0.01%	→	30,000 seeds (30 x 1,000 or 3 x 10,000)
0%	→	all seeds not practical & destructive

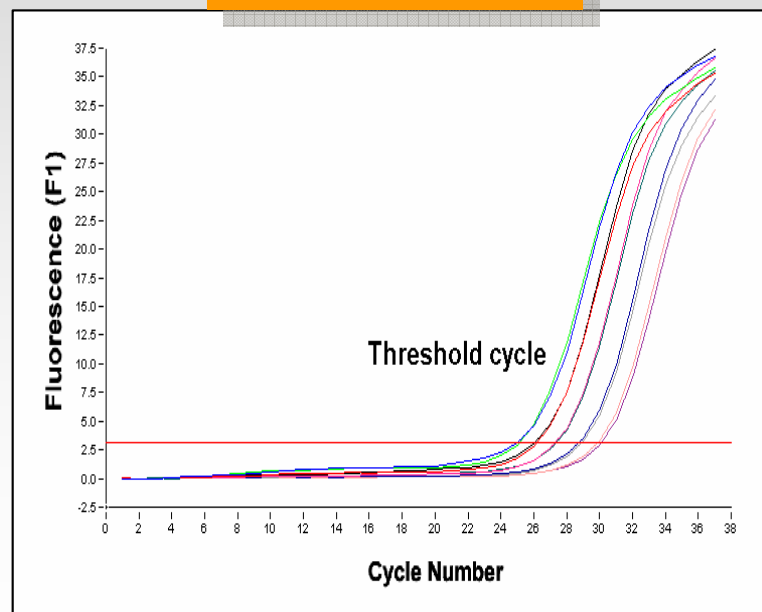
Analytical GM Seed / Grain Detection

- ⇒ Seed / grain sampling → representative sampling
- ⇒ Analyte extraction: particle size → homogenous distribution
- ⇒ Protein methods, e.g. immunological (ELISA) protein strips
- ⇒ DNA methods: Polymerase chain reaction (PCR)

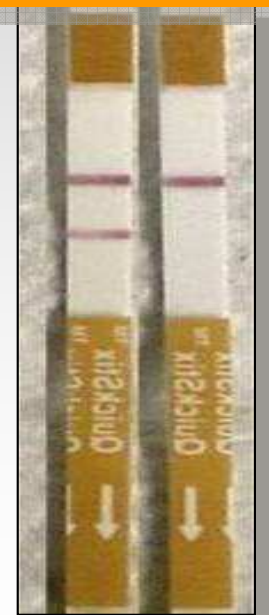
End-point PCR



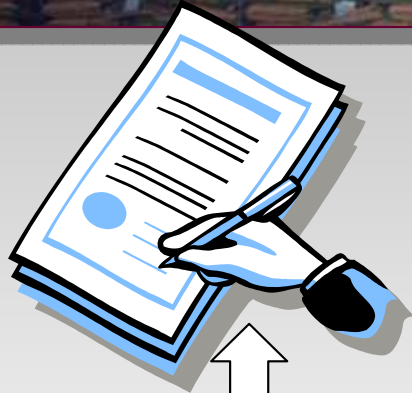
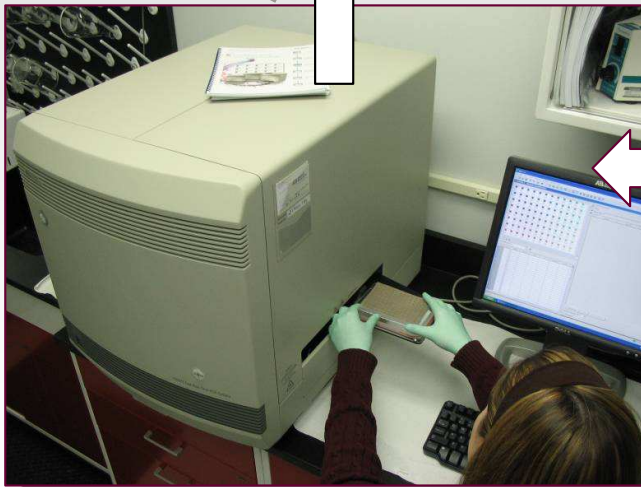
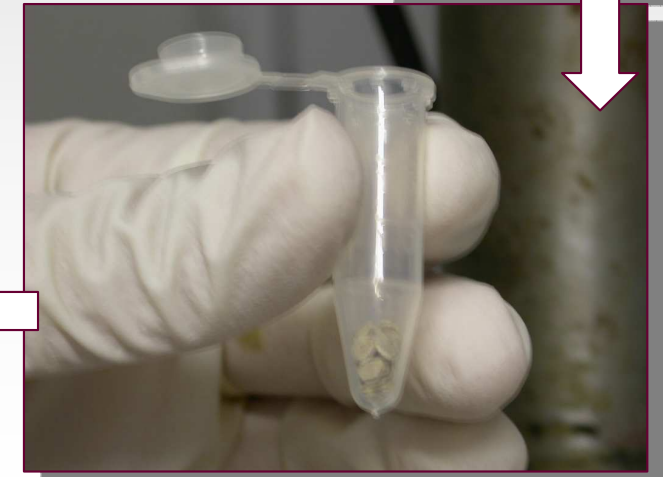
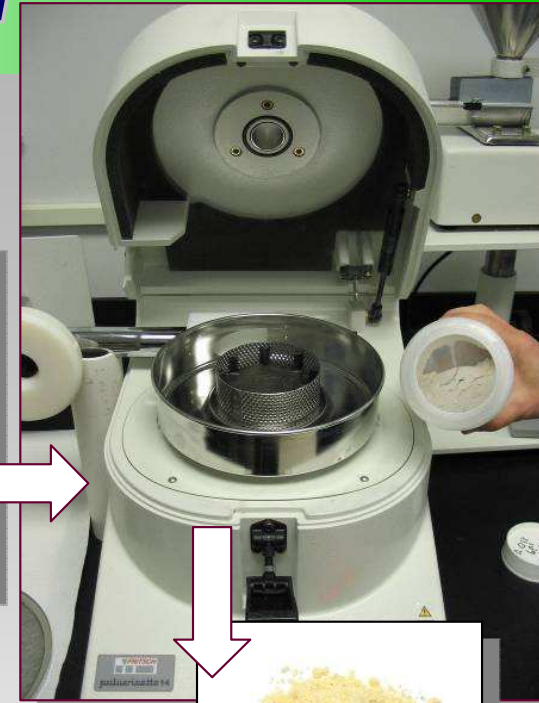
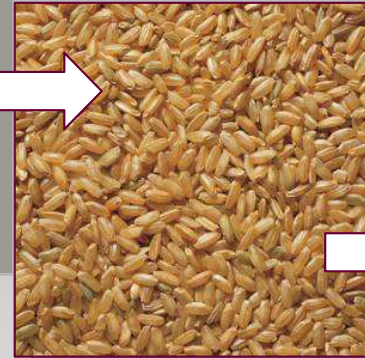
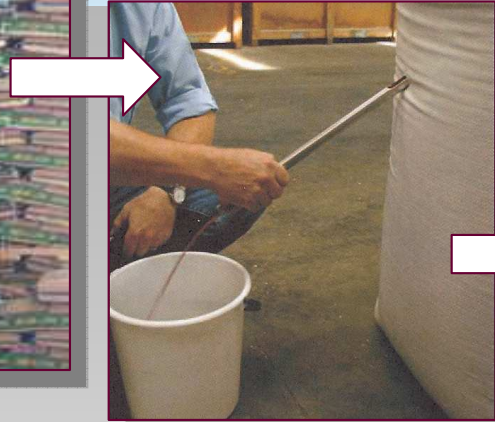
Real-time PCR



Protein strip

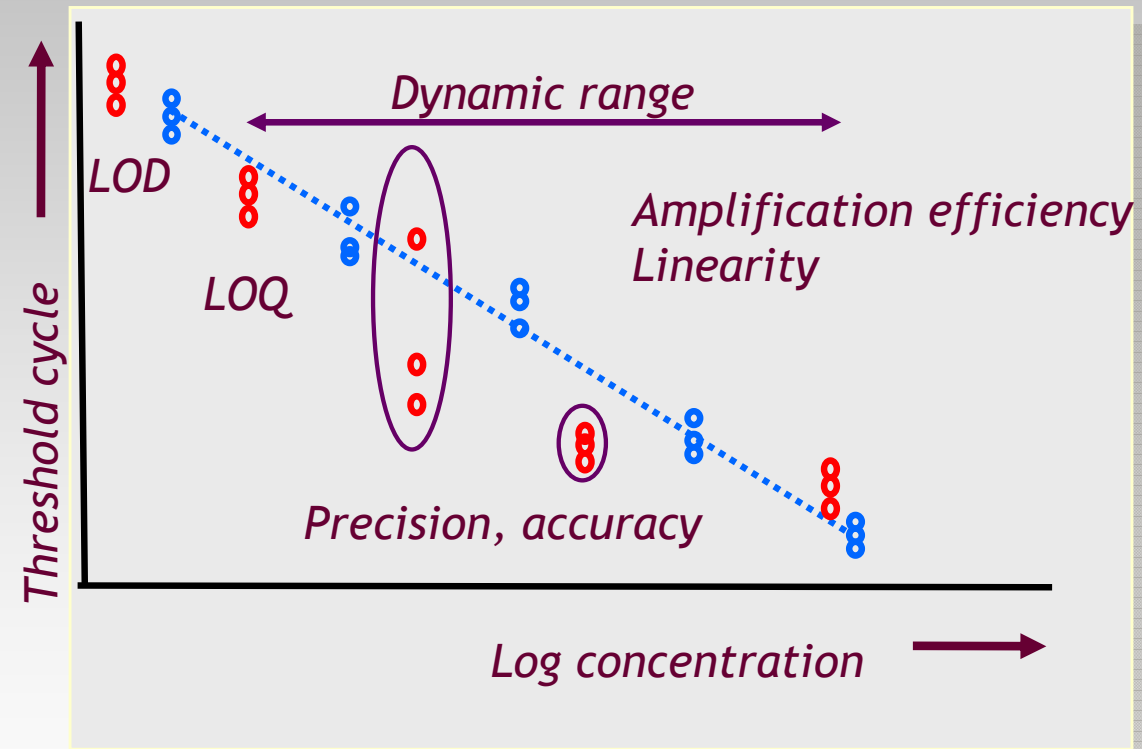
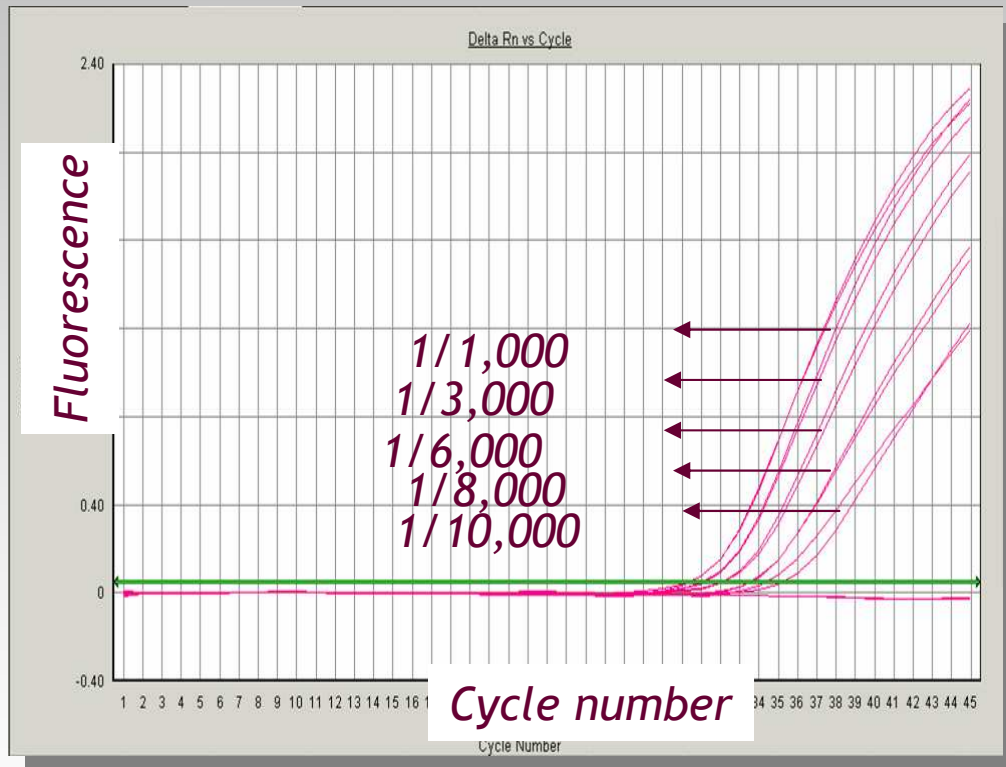


GM Seed Detection



Real-time PCR Validation

Earliest detected \rightarrow highest concentration of target



- Reference samples
- Blind samples

Semi-quantitative Sub-sampling



Qualitative tests on sub-samples used to generate quantitative results

Seedcalc (ISTA); Remund at al.; Laffont et. al. *Seed Science Research* (2005) 15, 197-204

3 sub-samples of 1,000 seeds each

PCR or Protein strip

Estimated Level

Lot impurity (P=95%)



not detected

<0.10% GM seeds



0.04% GM seeds

<0.20% GM seeds



0.11% GM seeds

<0.41% GM seeds

Unit-of Measurement

Methodology	# Samples	Output	Unit-of-expression
Protein strips ¹	single multiple	qualitative semi-quantitative ³	Present / Absent Seed %
End-point PCR ^{1, 2}	single multiple	qualitative semi-quantitative ³	Present / Absent Seed %
Real-time PCR ^{1, 2}	single multiple	qualitative quantitative semi-quantitative ³ quantitative ³	Present / Absent GM DNA % Seed % GM DNA %

¹Trait-specific; ²Event-specific;

³Seedcalc (ISTA); Remund et al. Laffont et. al. Seed Science Research (2005) 15, 197-204



Stacked GM Products

1 GM seed in total of 100 seeds = 1% GM seed?

Single seed with	Zygoty	Semi-quantitative Sub -sampling	Quantitative Real-time PCR
# of events / traits	heterozygous homozygous	GM Seeds / Grains %	GM DNA copy%
1 trait	Hybrid	1%	0.5% [0.4-0.6%]
	Open pollinated	1%	1 %
2 traits	Hybrid	1% or 2%?	1% [0.8-1.2%]
	Open pollinated	1% or 2%?	2%
3 traits	Hybrid	1% or 3%?	1.5% [1.2-1.8%]
	Open pollinated	1% or 3%?	3%

**“All GM seeds are transgenic,
but some of them are more transgenic than others”**



EuropaBio Position on Unit-of-Measurement:

http://www.europabio.org/relatedinfo/GBE_unit_of_measurement.doc

- ⇒ No single unit-of-measurement allows full consistency throughout the value chain*
- ⇒ Use of different units-of-measurement can be aligned with the regulations*
- ⇒ Allow flexibility in the choice of reliable & validated methodology*
- ⇒ Measuring the presence of multiple inserts presents technical issues*

Proficiency testing (ISO43)



- ⇒ To verify methods are reliable using conditions under which they will be used
- ⇒ To determine the competence of laboratories for the execution of methods

- ⇒ Need to ensure that method testing reflect the 'real-life' situation in which tests are carried out rapidly, on large seed bulks, and in a high-throughput mode
- ⇒ Method testing must be carried out on all aspects of the method:
 - grinding, DNA extraction, PCR

Modular approach to method testing

- ⇒ Labs will be able to use most method modules according to their routine procedures
- ⇒ This allows the detection method to be combined with
 - various sample preparations
 - various DNA extraction procedures
 - different types of equipment
- ⇒ Scoring is kept impartial by delivering 'blind' (unknown) samples

Reference Materials (ISO30-34)



- ⇒ Produced according to international standards & guidelines & may be certified
- ⇒ Made available globally for single events through a designated third-party source
- ⇒ Made available for all products that are commercially available
- ⇒ To provide to government agencies in a globally harmonized approach
- ⇒ To use in calibration, validation & proficiency testing of detection methods

Reference DNA directly from Bayer


1. Cotton: LLCotton25
2. Rapeseed: Ms1, Ms8, Rf1, Rf2, Rf3, T45, Topas19/2
3. Corn: T25
4. Rice: LLRICE62
5. Soy: A2704-12

Certified Reference DNA available through AOCS

<http://www.aocs.org/tech/crm/>

1. Cotton: LLCotton25
2. Rapeseed: Ms8, Rf3
3. Corn: T25
4. Rice: LLRICE62

Certified Reference Seed Flours In progress



AOCS Certified Reference Materials are available for U.S. \$175.00 each plus postage and insurance (in U.S. add \$20. Outside U.S. add \$60).

Please complete the following information: (street address is required for shipping)

Name _____
 Company _____
 Street Address _____
 City _____
 State/Province _____
 Postal Code _____
 Country _____
 Phone _____
 Fax _____
 E-mail _____

Form of Payment:
 • • Credit Card • • American Express • • MasterCard • • Visa

Expiration Date on Card _____
 Card Number _____
 Name as Printed on Card _____
 Signature _____

I would like to receive information on the following:

Official Methods and Recommended Practices of the AOCS, 5th Edition, 2nd printing
 Laboratory Proficiency Program
 AOCS Membership
 Physical and Chemical Characteristics of Oils, Fats, and Waxes, 2nd Edition

To contact AOCS Technical Services:
 Phone: +1-217-359-2344
 Fax: +1-217-351-8091
 E-mail: technical@aocs.org
 Web: www.aocs.org/tech

Return this form with payment to:
 AOCS Technical Services
 P.O. Box 3489
 Champaign, IL 61826-3489 USA
 Fax: +1-217-351-8091

Certified Reference Materials

Order Form • Invoice

Bayer Samples	Quantity
AOCS 0306-A Conventional Cotton Leaf DNA (10 µg)	
AOCS 0306-B Conventional Canola Leaf DNA (10 µg)	
AOCS 0306-C Conventional Corn Leaf DNA (10 µg)	
AOCS 0306-D Conventional Rice Leaf DNA (10 µg)	
AOCS 0306-E LLCotton25 Cotton Leaf DNA (10 µg)	
AOCS 0306-F Ms8 Canola Leaf DNA (10 µg)	
AOCS 0306-G Rf3 Canola Leaf DNA (10 µg)	
AOCS 0306-H T25 Corn Leaf DNA (10 µg)	
AOCS 0306-I LLRICE62 Rice Leaf DNA (10 µg)	
Number of Samples	
Subtotal (# of samples x U.S. \$175)	
Add Postage <small>(U.S. \$10; Outside U.S. +U.S. \$60)</small>	
Total Amount Due	

Samples will be sent after receipt of payment

Summary

Analytical GM Seed & Grain Detection

- ⇒ *Thresholds & Confidence limits → Setting standards*
- ⇒ *Method development & validation → International method criteria (e.g. LOD)*
- ⇒ *Type of detection tools → PCR, RT-PCR, Protein strips*
- ⇒ *Unit-of-measurements → DNA copies, Seed%*
- ⇒ *Products with stacked events → Quantitation?*
- ⇒ *Proficiency testing → Method modularity allows flexibility*
- ⇒ *Reference materials → International standards, availability*
- ⇒ *Harmonization through EuropaBio*



Bayer CropScience



Providing sustainable, high value plant-based solutions for agriculture, nutrition, health and biomaterials.

Thank you for your kind attention