Current Status of PCR Testing

AEIC
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Current Status of PCR Testing

CAVEAT EMPTOR!!

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Lack of Standardization

Sample

Split 1  Split 2

Lab A
Posiive

Lab B
Negative
Lack of Standardization

Sample

Split 1

Lab A

15% GMO DNA

Split 2

Lab B

2% GMO DNA

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Lack of Standardization

Sample

Split 1 Split 2

Lab A Lab B

15% GMO DNA

2% GMO DNA

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Lack of Standardization

What are meaningful results?

Highly Processed Samples:

...positive, but insufficient corn DNA to quantify

50% GMO DNA
(This could be 1 of 2 DNA molecules extracted)
Unexplained Variations
Inter-Lab & Intra-Lab

SOME POTENTIAL SOURCES:

♦ Sampling
♦ Sample prep
♦ Extraction
♦ PCR Routine
♦ Reference Materials
Unexplained Variations

Sampling:

♦ Different methods of sampling
♦ Size of sample taken
♦ Number of samples taken
♦ Mixing
♦ Contamination from Official Samples
Sampling: Representativity and Sensitivity

Increasing homogeneity / representativity

High sampling and subsampling effort

Potentially decreasing analytical sensitivity due to DNA removal

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Unexplained Variations

Sample Preparation:

♦ Different methods of splitting
♦ Different methods of grinding
♦ Variation in particle size
♦ Cross Contamination
Unexplained Variations

Extraction:

- Which procedure used
- Efficiency of extraction
- Alternative procedure available
Unexplained Variations

PCR Procedure:

♦ Machine specific variability
♦ Number of cycles
♦ Presence of inhibiting chemicals
♦ Specificity and validity of primer sets
Unexplained Variations

Reference Materials

♦ Copy numbers of inserted cassettes
♦ Polyploid status of chromosomes
♦ Similar behavior of reference & transgene
♦ Qualitative or quantitative
♦ General or specific events
♦ Is the reference material what it purports to be?
Unexplained Variations

Impact:

- Hinders commercial transactions
- Negative reflection on biotech as a useful application to the food chain
- Legal disputes and rejection of shipments

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Steps of Sample Preparation - Important Factors -

Representatvity, homogeneity

Extraction efficiency / yield
DNA integrity, purity

Sample → Sub sample → Analytical sample → Extracted DNA → Purified DNA

Sampling plan, number of samples
Grain size, sample size
Determination of DNA Concentration

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PCR Analysis of DNA
- Important Factors -

Aliquot of DNA solution → PCR → Detection of PCR-Products → Additional Verification

PCR sensitivity
PCR specificity

Absolute DNA input
Cycle-number, assay optimization, polymerase conc., inhibition- and negative-controls, etc
What Can AEIC Do?

Esoteric Discussions of Biotech in Foods

Commercial Applications and General Understanding of Biotech in Foods

A Forum to Bridge the Gap

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What Can AEIC Do?

A Forum for Scientific Expertise

Informal Transfer of Information

&

Formal Transfer of Information
Informal Transfer of Information

Membership Overlap

AEIC + AACC, IFT, ACS, AOAC, etc.

Conduit to translate technical information to “users” and to bring the needs of users back to “developers”

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Formal Transfer of Information

Provide a science based source of information to meet the technical needs of groups working to standardize testing of genetically engineered foods.

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Formal Transfer of Information

Develop position papers: e.g.,
*Effective sampling procedures*
*Minimum QA protocols*
Formal Transfer of Information

Technical Information: Test Guidelines

- Effective sampling procedures
- Minimum QA Protocols (for lab and field tests)
- Guide for selection of a test kit or a contract laboratory
- Limitations of the testing technologies available
Formal Transfer of Information

Public Voice for Technical Information

A non-biased source of public oriented information on biotechnical concerns

♦ Educators
♦ Regulators
♦ News media
♦ Legislators