Example, Status and experience from Brazil PART II

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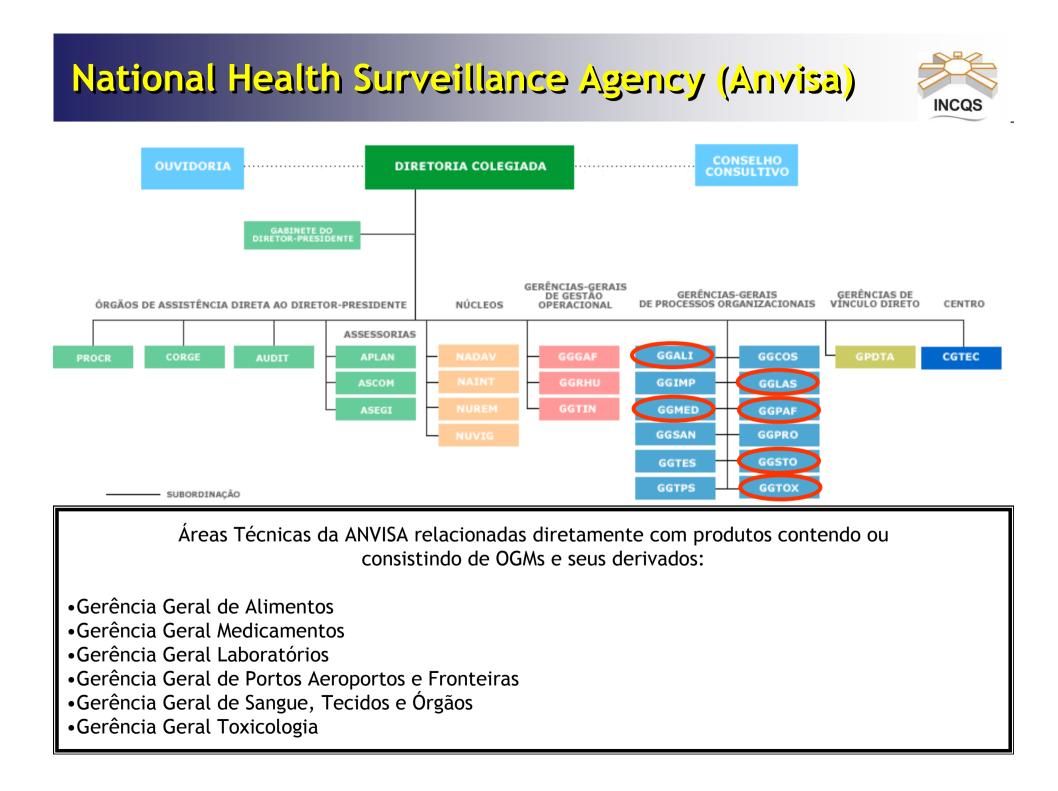Ministry of Health - Brazil

Foz do Iguaçu
4th-5th December 2009

INCQS

MINISTRO DA SAÚDE

SUBORDINAÇÃO
VINCULAÇÃO

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Conselho de Saúde Suplementar

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Hospital Fêmina S/A
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INCQS

National Health Surveillance Agency (Anvisa)

Áreas Técnicas da ANVISA relacionadas diretamente com produtos contendo ou consistindo de OGMs e seus derivados:

- Gerência Geral de Alimentos
- Gerência Geral Medicamentos
- Gerência Geral Laboratórios
- Gerência Geral de Portos Aeroportos e Fronteiras
- Gerência Geral de Sangue, Tecidos e Órgãos
- Gerência Geral Toxicologia

National Health Surveillance Agency (Anvisa)

- Stablishment of Technical Regulations regarding authorisations, registration and inspection of products for human use

- Financial support

- Assisting CTNBio together with other entities in defining biosafety assessment parameters for GMOs and their by-products

- Trainning of professionals to monitor the labelling of products containing or consisting of GMOs on the basis of documental traceability

- Stablishment of monitoring programes of food products to evaluate the compliance with labelling legislation

Oswaldo Cruz Foundation (Fiocruz)

National Institute of Quality Control in Health

National Institute of Quality Control in Health

Inaugurated in 1981 is a Technical Scientific unit of the Oswaldo Cruz Foundation – Fiocruz, acting as a National Reference Agency for technological and normative questions related to the quality control of products, environments and services linked to the Sanitary Surveillance System.

MAIN ACTIVITIES

- Evaluation of products such as food, drugs, vaccines, diagnostic reagents, blood derivatives, cosmetics, hygiene products, hemodialysis products;

- Technical support for the registration of vaccines and blood derivatives;

- Production of reference chemical and biological substances;

- Development of new analytical techniques;

- Contribution to the elaboration of the sanitary legislation

- *Stricto sensu* and *lato sensu* postgraduate course in sanitary surveillance

National Institute of Quality Control in Health

ISO/IEC 17025

INCQS was the first Official Laboratory of Quality Control in Health to have several assays and calibrations accreditated by the National Institute of Metrology, Standardization and Industrial Quality (Inmetro).

✓61 ASSAYS / CALIBRATIONS UNTIL 2009

✓GMO laboratory is initiating the process of accreditation according to ISO/IEC 17025

GMO Proficiency tests

- 2002 – 16 samples Roundup Ready soya in CRM (IRMM) – qualitative assay - **satisfatory performance**

- 2003- GeMMA GeM22 - Roundup Ready soya in sausage meat – qualitative assay – **satisfatory performance**

- 2004- GeMMA GeM27 – Roundup Ready soya in baked biscuit- qualitative assay – **satisfactory performance**

- 2005 – GeMMA GeM M15 – MON 810 maize in maize flour – qualitative assay – **satisfactory performance**

- 2007- GeMMA GeM SU04 (04 samples)- Roundup Ready soya in mixed flours-qualitative and quantitative assays – **satisfactory performance**

DESENVOLVIMENTO E VALIDAÇÃO INTERLABORATORIAL DE SISTEMAS DE DETECÇÃO E QUANTIFICAÇÃO EVENTO-ESPECÍFICOS DE OGMS NO BRASIL

Pesquisador Líder: Edna Maria Morais Oliveira
Instituição líder: Embrapa Agroindústria de Alimentos
Embrapa Soja
Embrapa Arroz e Feijão
UFSC
UFRS
INCQS

Brazilian commercial approvals

18 plants approved by National Biosafety Technical Comission (CTNBio)

Roundup Ready soybean (GTS 40-3-2), 1998

LibertyLink corn (Corn T25), 2007
Guardian corn (MON 810). 2007
Bt 11 corn. 2007
GA21 corn. 2008
Roundup Ready2 corn (NK 603), 2008
Herculex I corn (TC1507), 2008
MON 89034 corn, 2009
MIR 162 corn, 2009
MON810 x NK 603 corn, 2009
Bt11 x GA21 corn, 2009
TC1507 x NK 603 corn, 2009

Bollgard® cotton (531), 2005
Roundup Ready cotton (MON 1445), 2008
LibertyLink cotton (LL 25), 2008
Bollgard cotton (MON 15985), 2009
Widestrike cotton, 2009
MON531 x MON 1445 cotton, 2009

Brazilian commercial approvals

Events to be approved soon:

✓LibertyLink rice (LL 62 rice) – glifosinate ammonium tolerant

✓Soybean A5547-127 - glifosinate ammonium tolerant

✓Soybean tolerant to herbicide imidazolinone

✓ Which laboratorial system shall we have to analyze so many different events?

✓ Which methods should be used?

✓ How to determine the Measurements Uncertainty?

✓ How to differentiate the hybrids in a mixed product?

Why to Analyze GMO Food?

✓To verify the compliance with labelling legislation = Decree 4680 of April 24, 2003.

Foods or ingredients been intended for the human or animal consumption that contain or are produced from GMO above 1 %, must present this information on the label

✓To check the presence of the symbol that must compose the labels, established by the Ordinance 2.658 of December 26, 2003 (Ministry of Justice)

✓To guarantee the consumer's right of choice.

Why to Analyze GMO Food?

✓National / international trade of grains and raw materials.

✓To check of the presence of events not authorized by the Brazilian legislation.

✓Post-marketing surveillance of the presence / content of GMO in order to predict possible adverse effects in the long term.

Why to Analyze GMO Food?

Primary Production

Production Process

Wholesale and retail sector

Final Product

Focus of Inspection and Intervention

Technical Regulations

Food Quality Monitoring

Acquisition, storage and food handling

Consumer advice

INCQS

Verification of food products labelling

INCQS

ANVISA
•Tecnical regulations
•Financial Support
•Training
•National Monitoring programs

Ministry of Health (MS) is responsible for the enforcement of national legislation concerning labelling

State and Municipal Surveillance Centers
•Financial Support
•Planning and execution of surveillance actions
•Food establishments inspection
•Sampling

Official Analysis

OFFICIAL LABS
INCQS
• analysis
• training
• SOPs
• new methodologies
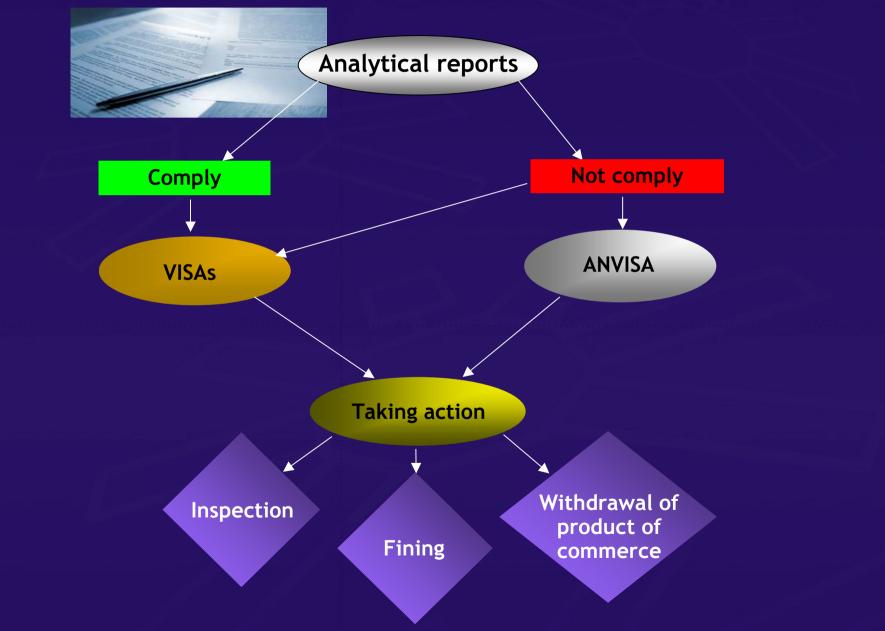
STATE LABS
(LACENs)-FUNED

Analytical Reports

Labelling food product verification

National Institute of Quality Control in Health

INCQS
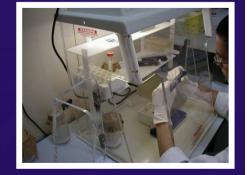
■ Food samples homogenization

▧ DNA extraction

■ ABI PRISM 7500

Analitical Methods based on:

✓GM Proteins detection – immunoassays:

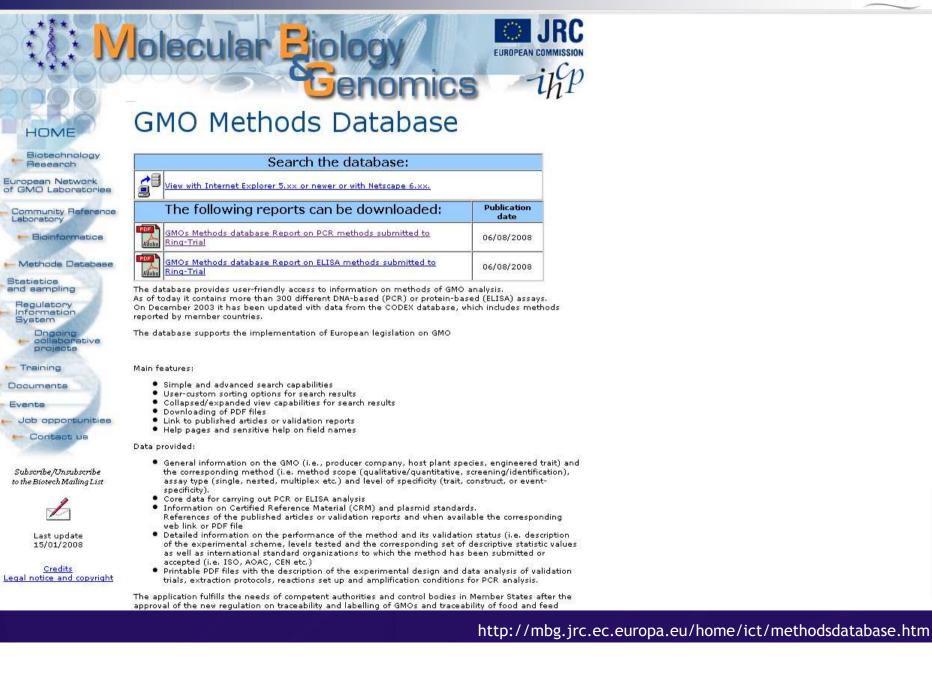Enzyme-Linked Immunosorbent Assay (ELISA)
Lateral flow test strips

✓Analysis of GM DNA sequences – Polymerase Chain Reaction (PCR)

Qualitative methods – P35S, t-nos, RR soybean, MON 810, Bt11, GA21 and Bt176

Quantitative analysis by Real Time PCR – TaqMan GMO Soy and Maize 35S Detection kit, Applied Biosystems – SDS 7500, AB

GMO Methods Database

INCQS

ENGL Steering Committee in collaboration with the ENGL Working Group Validation

"Definition of minimum performance requirements for analytical methods of GMO testing"

Version 13.10.2008

Phase 1: Evaluation of the method performance data submitted by the applicant as part of the official dossier; and

Phase 2: Evaluation of method performance data following a full validation study by collaborative trial.

FAO/ WHO Food Standards
CODEX alimentarius

"Proposed Draft Guidelines on Criteria for Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins, in Particular in Foods Derived from Modern Biotechnology"

(Dra. Shirley Abrantes - coordination)

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Detection of GMO in food products in Brazil: the INCQS experience

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Fernanda P. da Cruz [b,1], André L. Gemal [b,2]

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Table 3
Products analyzed for the presence of CaMV 35S and *nos*-terminator

Products	Number of samples	Positive samples		Positive samples
		35S1/S2	CF3/CR4	HA-*nos* 118-f/HA-*nos* 118-r
Powdered soy milk	13	6	6	6
French fries	1	0	0	0
Cookies	3	0	0	0
Texturized soy protein	1	0	0	0
Pasta	2	1	1	1
Sausages	5	2	2	2
Soy beverages	7	0	0	0
Raw soybeans	15	1	1	1
Products of maize	5	0	0	0
Canned soup	2	0	0	0
Dehydrated soup	17	10	8	4
Pet food	6	2	2	2
Canned corn	1	0	0	0
Total	78	22	20	16

Table 4
Detection of RR soybean, Bt 176 maize and MON 810 maize

Products	RR soybean	Bt 176 maize	MON 810 maize
Soy milk	6/6[a]	0/0	0/0
Pasta	1/1	0/1	0/1
Sausages	2/2	0/0	0/0
Raw soybeans	1/1	0/0	0/0
Dehydrated soup	4/10	0/5	0/5
Pet food	2/2	0/2	0/2
Total	16/22	0/8	0/8

[a] GM samples/examined samples.

Published papers

Soja geneticamente modificada em alimentos contendo farinha e preparados à base de farinha de trigo. Detecção e adequação à legislação de rotulagem

Genetically modified soybean in food containing wheat flour and wheat flour-based preparations. Detection and fitness to labeling legislation

Autores | Authors

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✉ *Autor Correspondente | Corresponding Author*

Resumo

A farinha de trigo é considerada como um dos principais ingredientes utilizados no preparo dos alimentos e, portanto, presente em praticamente todas as refeições dos brasileiros.

Em 2000, o consumo *per capita* de farinha de trigo correspondia a 53 kg e estava distribuída da seguinte forma no mercado brasileiro: 47% padarias; 14% indústrias de massas; 20% consumo doméstico; 8% indústria de biscoito; 5% indústria de pães; e 6% em outros segmentos. O objetivo deste trabalho foi avaliar a presença de soja e soja Roundup Ready® em amostras de farinhas de trigo e de preparados à base de farinha de trigo para a alimentação humana e verificar a adequação dos rótulos frente à legislação pela análise quantitativa por PCR em Tempo Real. O INCQS/FIOCRUZ analisou 16 amostras de farinhas de trigo de 10 marcas diferentes. Quinze (15) amostras apresentaram resultado positivo para o gene *le1* da lectina, demonstrando a presença de soja nestes produtos, e doze (12) amostras apresentaram resultado positivo para o gene CP4 EPSPS, específico da soja RR®, evidenciando a utilização de soja geneticamente modificada nestes produtos. Todas as amostras analisadas denominadas como preparados à base de farinha de trigo para a alimentação humana, conhecidos comercialmente como mistura para bolos, quiches, panquecas e bolinhos de chuva apresentaram resultados positivos para o gene específico da soja RR® e deveriam conter no rótulo o símbolo estabelecido na Portaria n° 2658, de 22 de dezembro de 2003.

Palavras-chave: *Soja geneticamente modificada; Alimentos; PCR; Trigo; Vigilância sanitária.*

Summary

Published papers

Tabela 1. Amplificação, identificação e quantificação das amostras analisadas.

Amostra (Nº)	Lectina positivo	Soja RR® positivo	Soja RR® acima de 1%
Farinha de Trigo (14)	13	12	12
Farinha de Trigo Integral (2)	2	0	0
Mistura para bolo (5)	5	5	5
Mistura para quiche (1)	1	1	1
Mistura para panqueca (1)	1	1	1
Mistura para bolinho de chuva (1)	1	1	1
Total (24)	23	20	20

Title: EVALUATION OF COMPLIANCE WITH LABELING LEGISLATION IN FOODS CONTAINING ROUNDUP READY SOY IN BRAZIL

Corresponding Author: Ms Maria Regina Branquinho, M.D.

Corresponding Author's Institution: Instituto Nacional de Controle em Qualidade em Saúde

First Author: MARIA REGINA BRANQUINHO, M.Sc.

Order of Authors: MARIA REGINA BRANQUINHO, M.Sc.; Maria Regina Branquinho, M.D.; Renata Trotta B Ferreira, MSc; Paola Cardarelli-Leite, PhD; Maria Regina Branquinho, MSc; RENATA T FERREIRA, M.Sc.

Table 1 - Main characteristics of the calibration curves

Run	R^2	Intercept	Slope	Efficiency	Range ΔCt
1	0.9953	7.6075	-3.5953	89.7%	4.94-11.06
2	0.9954	7.4419	-3.5560	91.0%	4.89-11.00
3	0.9989	6.6794	-3.3650	98.2%	4.31-10.04
4	0.9990	7.4185	-3.3806	97.6%	4.99-10.77
5	0.9964	7.6481	-3.6522	87.8%	5.28-11.42

Table 2. Reproducibility of the Ct measurements of replicate standards from 0.1 to 5% transgenic content

Quantity	Mean Ct run 1	Mean Ct run 2	Mean Ct run 3	Mean Ct run 4	Mean Ct run 5	Mean Ct	SD[a]	CV[b] (%)
endogenous								
0.1%	24.75	24.77	24.55	24.66	23.06	24.36	0.73	3.00
0.5%	24.97	24.77	24.93	24.57	22.79	24.41	0.92	3.75
1%	24.87	24.48	24.86	24.5	22.78	24.30	0.87	3.58
2%	24.88	24.85	24.74	24.56	22.78	24.36	0.89	3.67
5%	24.92	24.93	24.89	24.55	22.73	24.40	0.95	3.89
transgenic								
0.1%	36.37	35.61	35.22	35.2	35.54	35.58	0.47	1.33
0.5%	34.26	33.14	32.84	32.67	33.02	33.18	0.63	1.89
1%	33.32	32.36	31.8	31.79	32.21	32.29	0.62	1.93
2%	32.23	31.33	30.98	30.85	31.13	31.30	0.55	1.75
5%	30.74	29.8	29.53	29.28	29.46	29.76	0.58	1.94

[a]SD= standard deviation; [b]CV (%)= coefficient of variation
Each value corresponds to the mean of three reactions.

Table 3 - Accuracy data for quantitative analyses.

CRM (%)	Ext.1 (%)	Ext.2 (%)	Ext. 3 (%)	Ext. 4 (%)	Mean (%)	SD[a]	CV[b] (%)	Bias[c] (%)
0.1	0.10	0.11	0.13	0.09	0.11	0.017	15.4	7.5
0.5	0.60	0.56	0.39	0.37	0.48	0.117	24.4	-4.0
1	1.20	0.76	0.84	1.09	0.97	0.207	21.3	-2.7
2	1.95	1.56	1.93	2.13	1.89	0.240	12.7	-5.4
5	4.19	5.76	4.85	4.75	4.89	0.650	13.3	-2.2

[a]SD=standard deviation; [b]CV (%)=coefficient of variation; [c]Bias (%)=((mean value-true value) / true value) x 100.
Each value corresponds to the mean of three reactions.

Table 4 – Amplification data used to determine the absolute and relative detection and quantification limits.

				L.O.Q.		L.O.D.	
Estimated RR soybean copies per PCR reaction	4425	885	221	44	22	11	5.5
Estimated %RR soybean per PCR reaction	5	1	0.25	0.05	0.025	0.0125	0.00625
no. positive/no. reactions	12/12	12/12	12/12	12/12	12/12	12/12	6/12
Mean Ct-values of positives	30.88	33.29	35.49	38.04	38.90	39.36	-
SD of Ct-values	0.241	0.269	0.397	0.600	0.768	0.714	-

SD=standard deviation

Table 5 – Food samples analyzed and transgenic content

Food products	Number of samples analyzed	Number of samples positive for RR soybean	Number of samples with transgenic content <1%	Number of samples with transgenic content >1%
Raw soybeans	47	12	5	7
Soy beverages	66	5	5	0
Powered soy milk	15	6	6	0
Infant formula	14	7	6	1
Texturized soy protein (TSP)	11	8	5	3
Soy flour	7	3	1	2
Soy meal	7	7	0	7
Soy fiber	2	2	2	0
Dehydrated soup	23	6	2	4
Meat products	20	7	7	0
Vegetal products	9	4	4	0
Pasta	5	0	0	0
Snacks and cookies	7	0	0	0
Pet food	7	1	0	1
TOTAL	240	68	43 (63.2%)	25 (36.8%)

January 2008

Labelling of GM containing Food

Technical Regulations for Labelling
of Packaged Foods
RDC 259, 20/09/2002

"Organic" or "Natural"
Law No. 10,831, 23/12/2003

UTILIZAÇÃO DO GRÁFICO DE SHEWHART COMO FERRAMENTA ANALÍTICA

NO CONTROLE DA QUANTIFICAÇÃO DE SOJA GENETICAMENTE

MODIFICADA EM ALIMENTOS POR PCR EM TEMPO REAL

SHEWHART CONTROL CHART FOR ANALYTICAL TOOL IN THE QUANTIFICATION OF GENETICALLY MODIFIED SOYBEAN IN FOOD BY REAL TIME PCR

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2009 / 2010 ??

In preparation...

- **SOJA RR® EM BEBIDAS À BASE DE SOJA. DETECÇÃO E APLICABILIDADE DA LEGISLAÇÃO DE ROTULAGEM -** Paola Cardarelli-Leite, Renata Trotta Barroso Ferreira & Maria Regina Branquinho – *Alimentos e Nutrição – Araraquara*, 2010

- **REAL-TIME PCR MULTIPLEX METHOD FOR THE QUANTIFICATION OF ROUNDUP READY SOYBEAN IN RAW MATERIAL AND PROCESSED FOOD -** Maria Regina Branquinho, Renata Trotta Barroso Ferreira & Paola Cardarelli-Leite – *JFCA*, 2010

- **DETECTION OF GENETICALLY MODIFIED ORGANISMS IN FOOD: CRITICAL POINTS AND COMPARISON AMONG TWO DIFFERENT DNA EXTRACTION METHODS -** Maria Regina Branquinho Renata Trotta Barroso Ferreira & Paola Cardarelli-Leite – *TRENDS in Biotechnology*, 2010

Challenges

✓Development of methods for simultaneous detection and quantification of various GMOs including unauthorized ones

✓"Unknown"

✓Search on processed products

Muito Obrigada!