

***Relevancia de virus entéricos en productos de IV gama:
detección y medidas de control***

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Consumidor

- ✓ *Conciencia nutricional*
- ✓ *Alimentos sanos, frescos y de alta calidad*
- ✓ *Comidas de fácil preparación*
- ✓ *Menos aditivos y conservantes*
- ✓ *Alimentos seguros*



Productos de IV gama



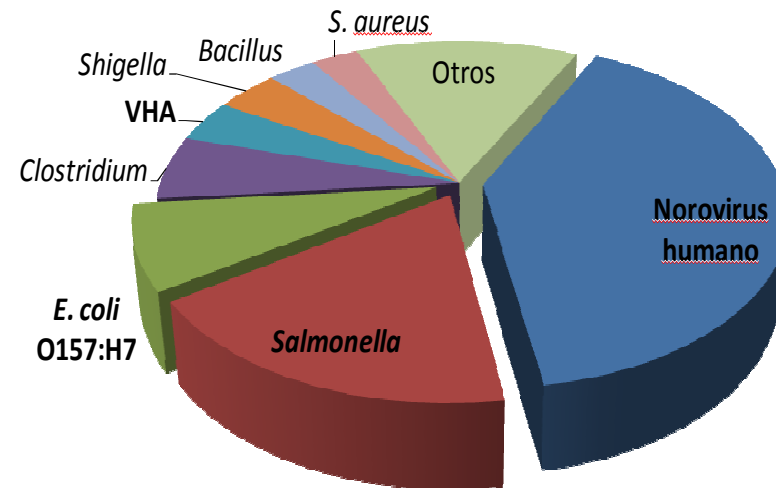
- ✓ **70,6 Millones de kilos (2010)**
- ✓ **Incremento 6%**
- ✓ **200 M€**
- ✓ **2% frutas de IV gama**

Productos de IV gama



Brotes alimentarios asociados a vegetales en IV gama

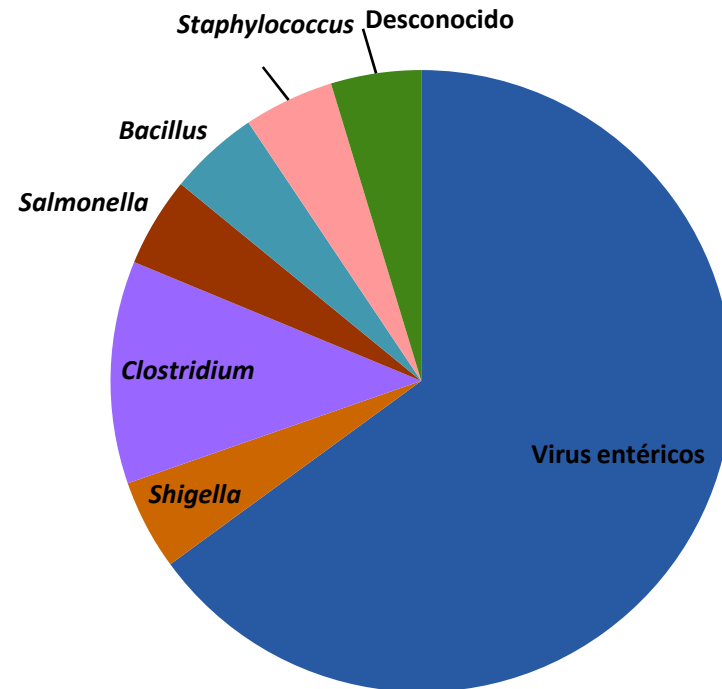
✓ 13 % de los identificados (origen, patógeno) USA (1990-2005)



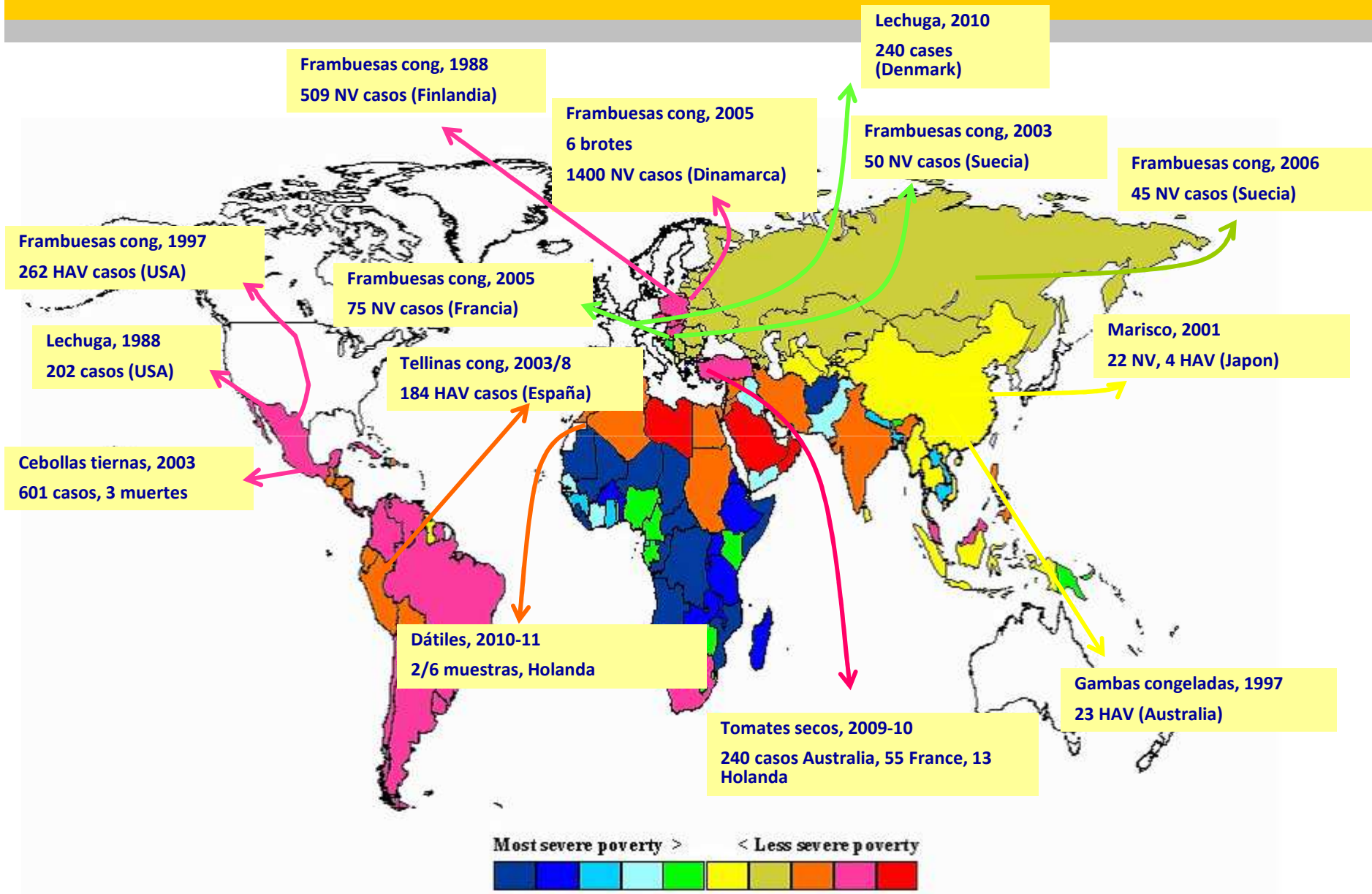
Productos de IV gama



Brotes alimentarios asociados a vegetales en IV gama



Brotos relacionados con la importación de alimentos



Self-Organizing Map Clusters based on 10 poverty indicators and GNP per capita



Métodos analíticos

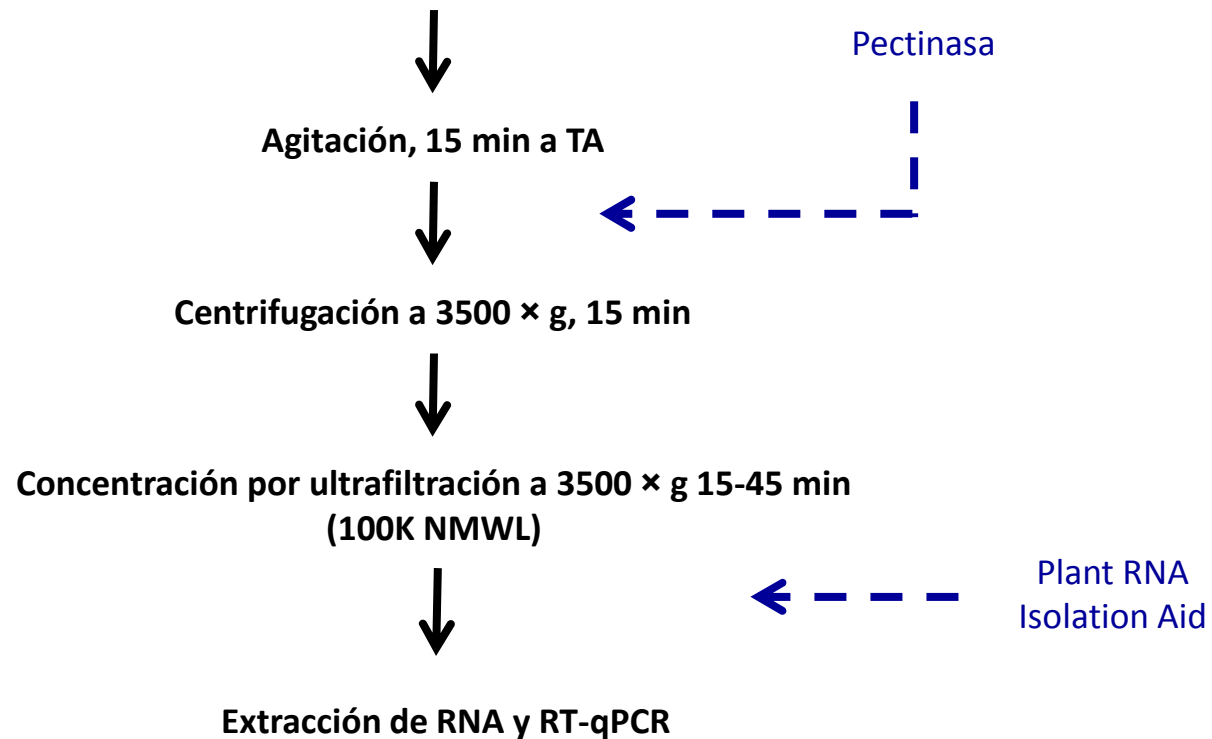


Método para fruta y hortalizas



15 g

60 mL tampón de elución (50 mM Glicina, 100 mM Tris, 1% extracto de carne) a pH 9,5



Validación método para fruta y hortalizas

	Nº positivos/Nº muestras analizadas										
	VHA (TCID ₅₀ /15g)				NV (PCRU/15g)			RV (TCID ₅₀ /15g)			
	120	12	1,2	0,1	540	54	5,4	2,1	0,21	0,02	
Arándanos congelados	3/3 (35,5)	3/3	3/3	0/3	3/3 (17,9)	3/3	2/3	3/3 (14,0)	3/3	3/3	
Fresas	3/3 (2,1)	3/3	2/3	0/3	3/3 (1,7)	2/3	0/3	3/3 (2,8)	3/3	0/3	
Frambuesas	3/3 (37,5)	3/3	2/3	1/3	3/3 (19,6)	3/3	3/3	3/3 (3,4)	3/3	0/3	
Frambuesas congeladas	3/3 (4,4)	3/3	2/3	0/3	3/3 (2,6)	2/3	1/3	3/3 (1,7)	3/3	0/3	
Mezcla de bayas azuc.	3/3 (9,3)	3/3	2/3	0/3	3/3 (5,1)	3/3	1/3	3/3 (0,5)	3/3	3/3	
Lechuga	3/3 (83,1)	3/3	3/3	3/3	3/3 (43,9)	3/3	3/3	3/3 (2,9)	3/3	3/3	
Albahaca	3/3 (52,3)	3/3	3/3	2/3	3/3 (11,5)	3/3	3/3	3/3 (5,1)	3/3	3/3	
Menta	3/3 (51,6)	3/3	3/3	0/3	3/3 (46,3)	3/3	1/3	3/3 (21,2)	3/3	3/3	
Cebollas tiernas	3/3 (9,8)	3/3	3/3	0/3	3/3 (9,5)	3/3	1/3	3/3 (1,6)	3/3	3/3	
Perejil	3/3 (12,6)	3/3	3/3	0/3	3/3 (13,0)	3/3	2/3	3/3 (2,9)	3/3	3/3	

Método para fruta y hortalizas



25 g

40 mL tampón de elución (50 mM Glycine, 100 mM Tris, 1% extracto de carne) a pH 9,5



Agitación, 20 min a TA

Pectinasa



Centrifugación a 10000 × g, 30 min



Concentración 10% PEG 0,3 M NaCl



Extracción de RNA y RT-qPCR



European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Food and Nutrition

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Methods for the Analysis of Parasites, Viruses and Other Foodborne Pathogens

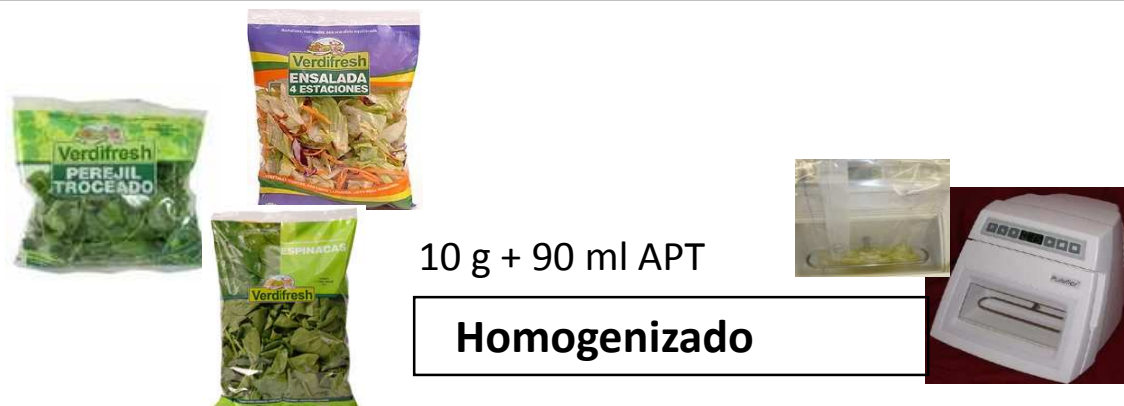
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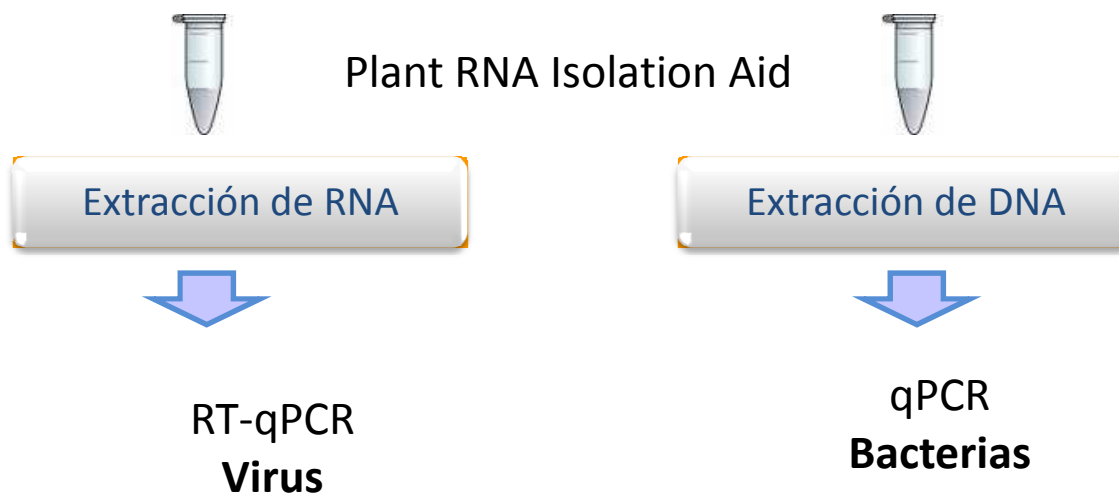
Identification Number	Title	Date Issued
Disclaimer	Disclaimer	January 2005
Laboratory Procedures		
OPFLP-01	Concentration of Norovirus Genogroups I And II from Contaminated Oysters and Their Detection Using the Reverse-transcriptase Polymerase Chain Reaction	February 2006
	Figures	February 2006

- Marisco
- Agua embotellada
- Cebollas tiernas

Recuperación simultánea de bacterias y virus



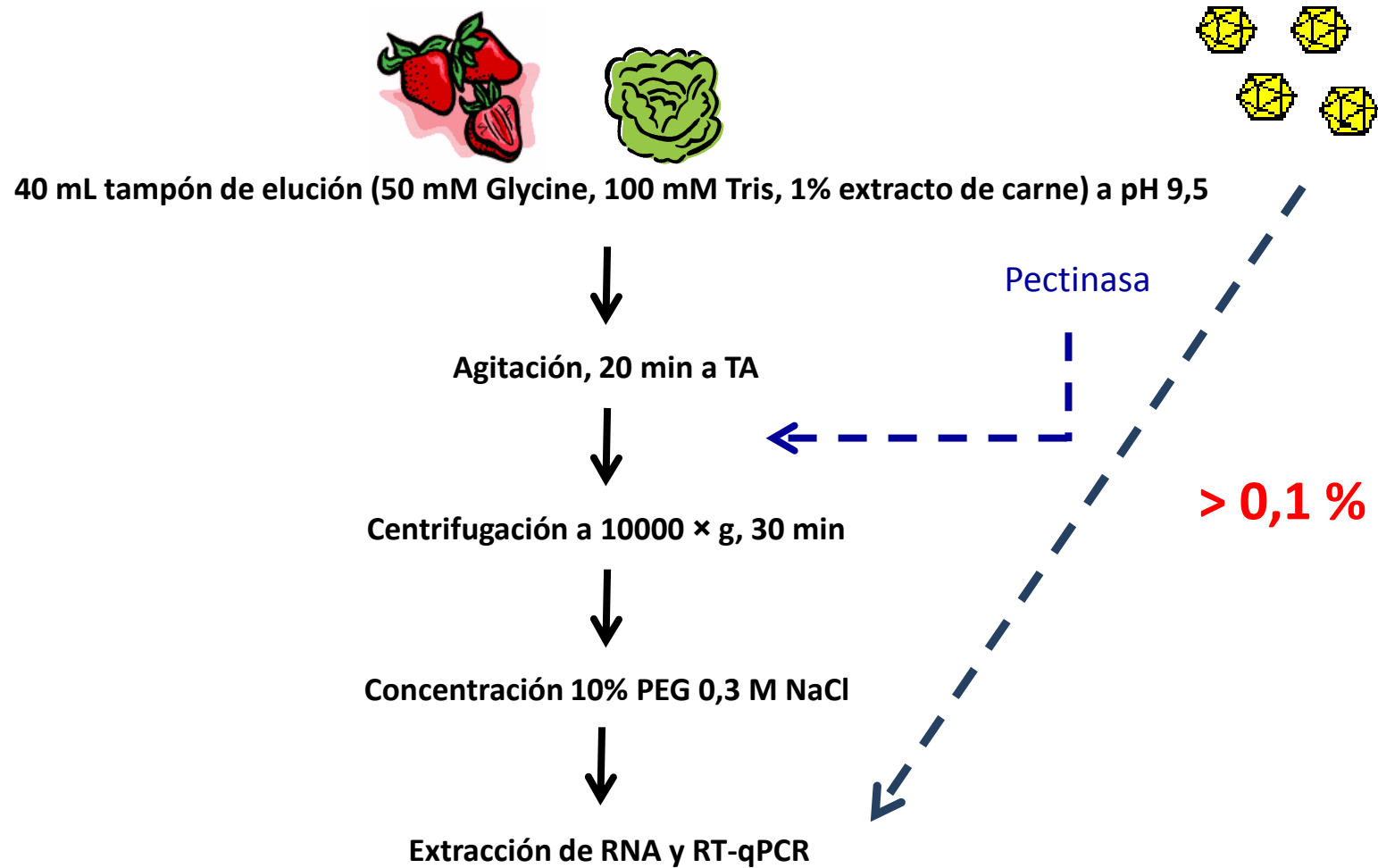
Precipitación con **PEG/NaCl** 1 h, 4 °C
Centrifugación, 30 min, 10.000 xg 4 °C



Recuperación simultánea de bacterias y virus

	% Recuperación		% Recuperación
Bacterias	Rango 10^6 - 10^3 ufc/g	Virus	Rango 10^5 - 1 TCID ₅₀ /g
<i>E. coli</i> O157:H7	57,4	NoV GII	9,2
<i>L. monocytogenes</i>	64,5	MNV	43,5
<i>Salmonella</i>	64,6	VHA	20,7

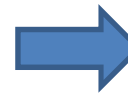
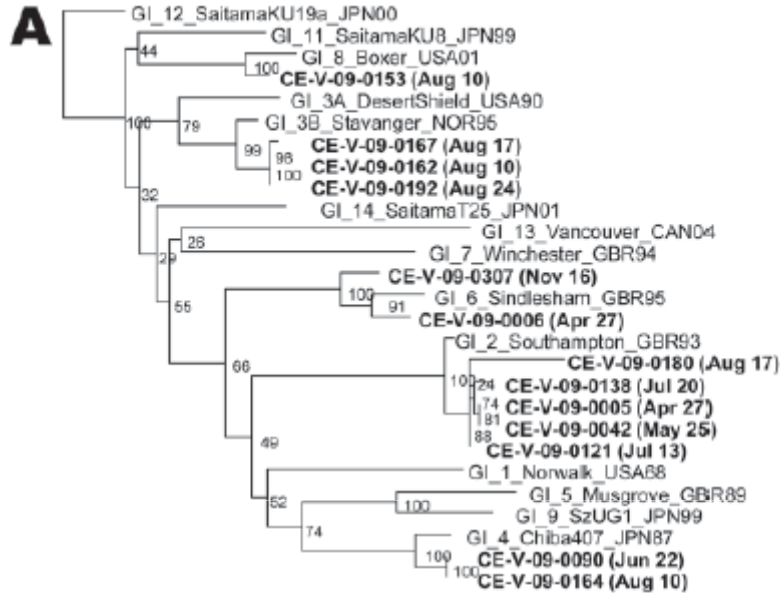
Control de proceso



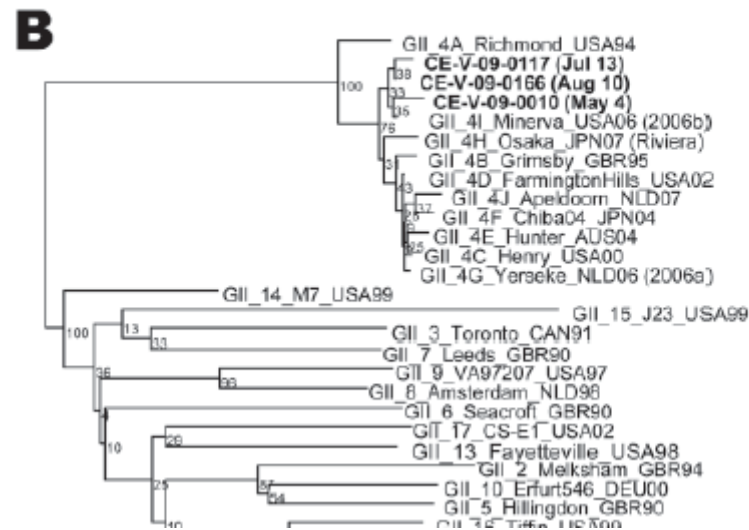
Incidenca



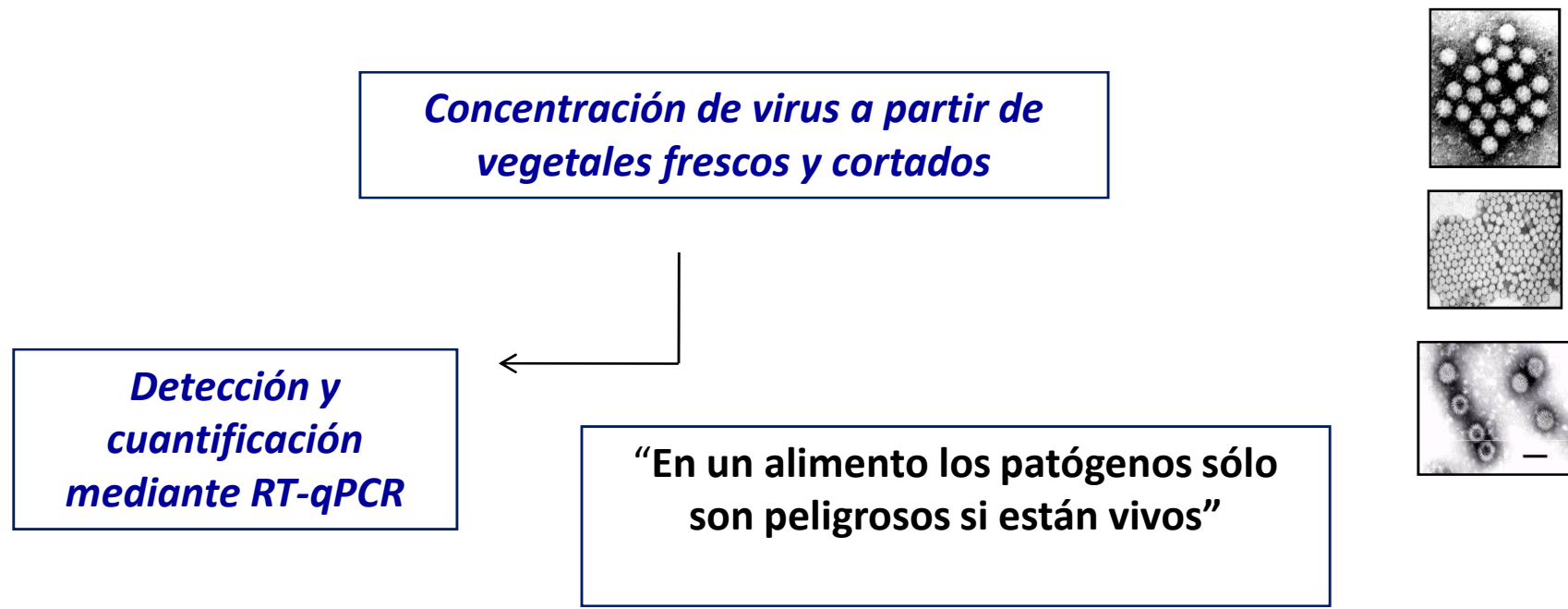
Ca
Fr
Be



RASFF
2010.0081

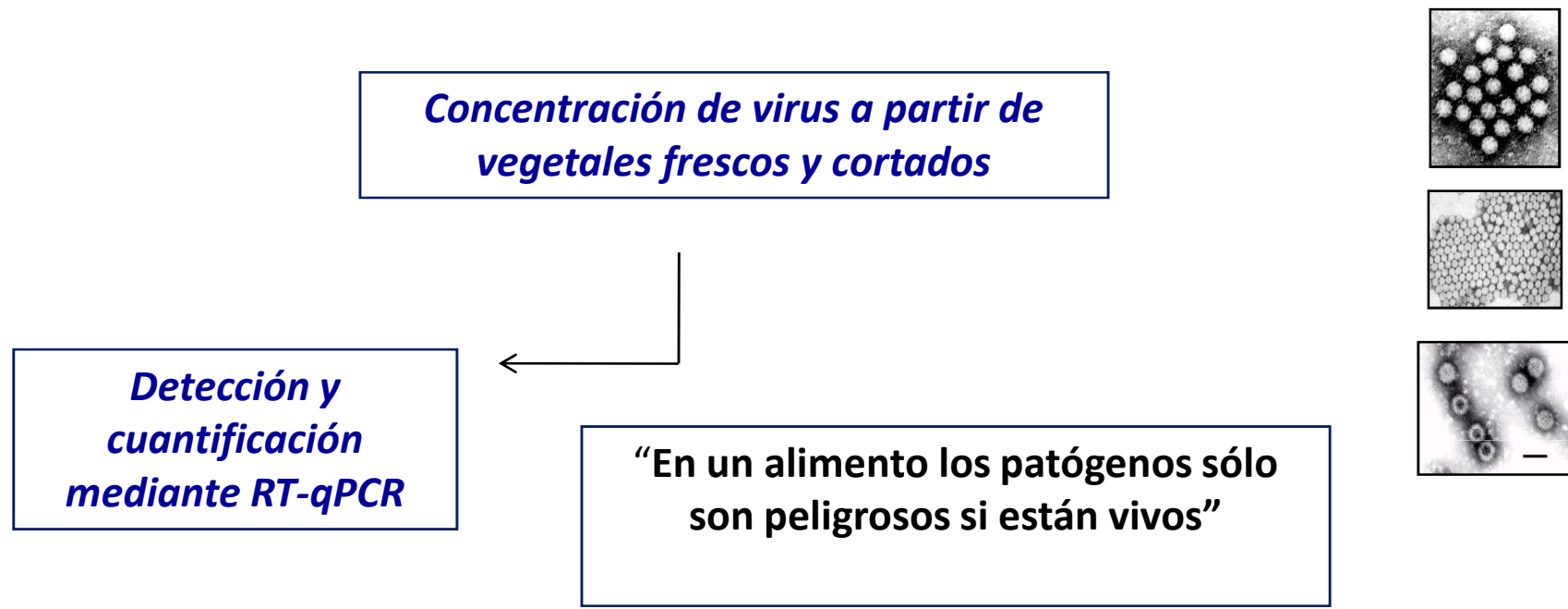


Detección de virus infecciosos



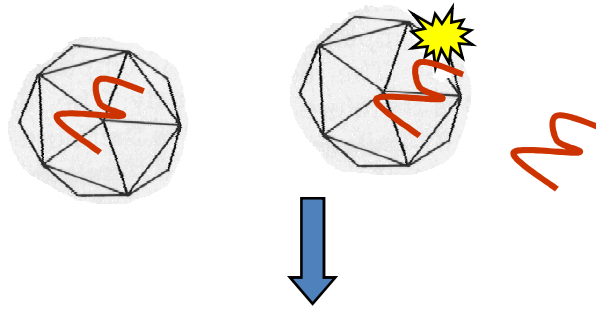
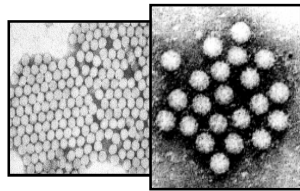
- Detección de fragmentos grandes
- CC + RT-PCR
- PMA
- Proteinasa K y RNasa

Detección de virus infecciosos

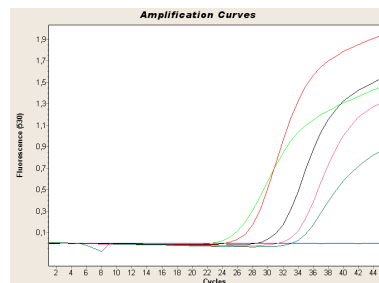
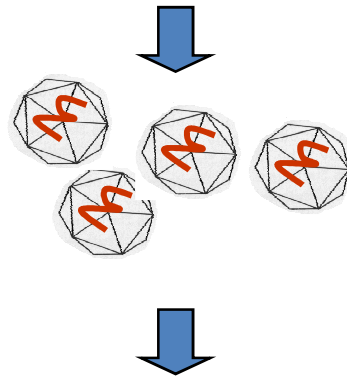


- Detección de fragmentos grandes
- CC + RT-PCR
- PMA
- Proteinasa K y RNasa

Proteinasa K y RNasa



Proteinasa K + RNasa



Lamhoujeb et al., 2008; Topping et al., 2009

PMA/EMA

Propidio de monoazida (PMA)

Etidio de monoazida (EMA)

