ILSI Europe
International Life Sciences Institute

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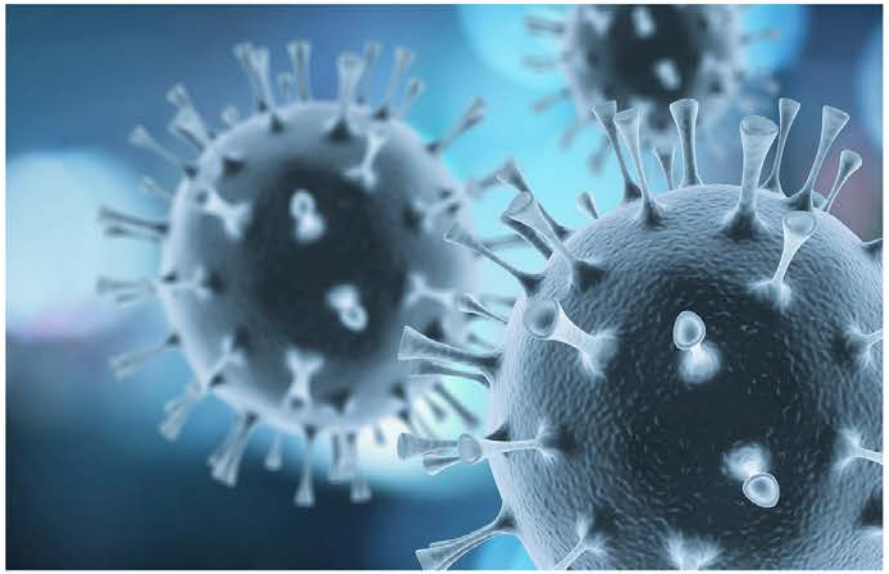
Webinar

Foodborne Viruses: Detection, Risk Assessment and Control Options in Food Processing

Organised by the *Microbiological Food Safety* Task Force



12 November 2019
16.00-17.00 CET, 9.00-10.00 EST


International Association for Food Protection®

Webinar Housekeeping

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Webinar Housekeeping

- It is important to note that all opinions and statements are those of the individual making the presentation and not necessarily the opinion or view of IAFP.

- This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

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We put relevant people
together to agree on common
scientific needs

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International Life Sciences Institute

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between academia, industry, public sector

Identifying and tackling existing and
emerging challenges in food and nutrition

Developing Science of highest quality & integrity

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> 430
Publications

52
Industry companies

38
expert groups

25,820
citations

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> 1000
Voluntary scientists

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Microbiological Food Safety Task Force

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ILSI Europe

ILSI EU Expert Working Group

- Organized by ILSI EU – first meeting 25 June 2015, Brussels
- Consisted of researchers (7) and food industry (7)
- Activities funded by the Microbiological Food Safety Task Force and Emerging Microbiological Issues Task Force

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Dr. Elissavet Gkogka – Arla Foods (Denmark)
Dr. Fabienne Hamon – bioMérieux Industry (France)
Prof. Alvin Lee – Institute for Food Safety and Health (USA)
Dr. Soizick Le Guyader – IFREMER (France)
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Prof. Marcel Zwietering – Wageningen University (Netherlands)

Dr. Mette Myrmel – Norwegian School of Veterinary Science (Norway)
Dr. Trevor Phister – PepsiCo Europe (UK)
Dr. Anna Charlotte Schultz – Technical University of Denmark (Denmark)
Dr. Anett Winkler – Cargill (Germany)
Dr. Sophie Zuber - Nestlé (Switzerland)
Dr. Annette Sansom – Campden BRI (UK)
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Review

Foodborne viruses: Detection, risk assessment, and control options in food processing

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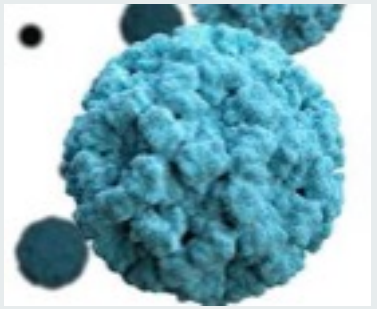
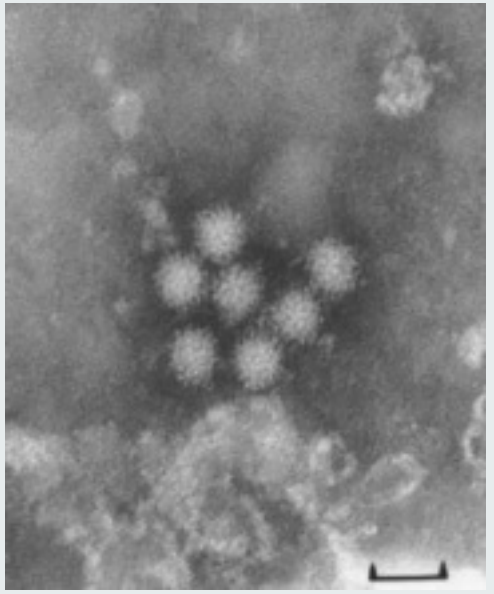
IJFM (2018) 285:110-128

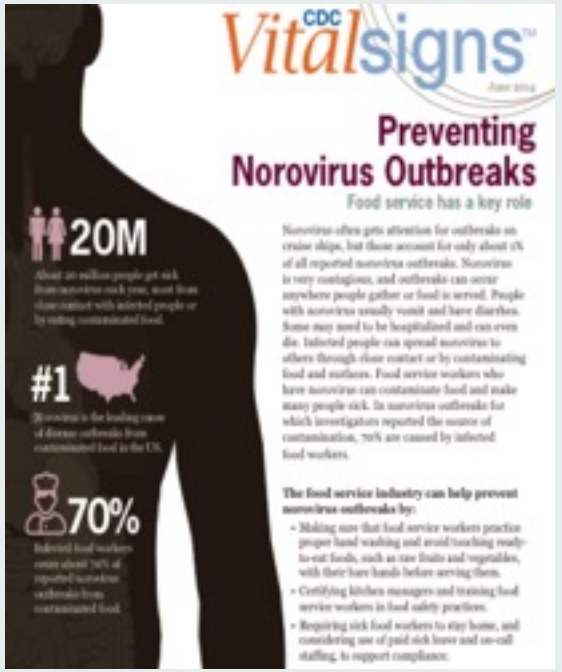
Why Viruses





- Frequent and under-recognized cause
- Ingredients and finished products are affected
- Global trade that impact multiple countries
  - HAV frozen berries from Canada, Serbia and Poland with cases in Italy
  - NoV in frozen strawberries from China affecting 12,000 in Germany
  - 2018 Winter Olympics

- Interpretation of positive detection
- Effective controls measures throughout food chain



Agenda

Housekeeping and Introduction

Tamara Ford, IAFP; Dr. Angeliki Stavropoulou, ILSI Europe and IIT-IFSH, Alvin Lee

Pros and Cons of Available Methods for Foodborne Virus Detection

Dr. Fabienne Hamon, bioMérieux, France

Translating Risk Assessment of Viruses into Practice

Dr. Elissavet Gkogka, Arla Foods, Denmark

Effect of Processing Technologies to Control Viruses in Foods

Dr. Sophie Zuber, Nestlé Research Center, Switzerland

Future Challenges and Gaps

Dr. Alvin Lee, Institute for Food Safety and Health, USA

Q&A after all speakers and please submit questions using the chat box

Pros and Cons of Available Methods for Foodborne Virus Detection

Fabienne HAMON, PhD.

RD molecular biology manager

IAFP/ILSI webinar, november 12th, 2019

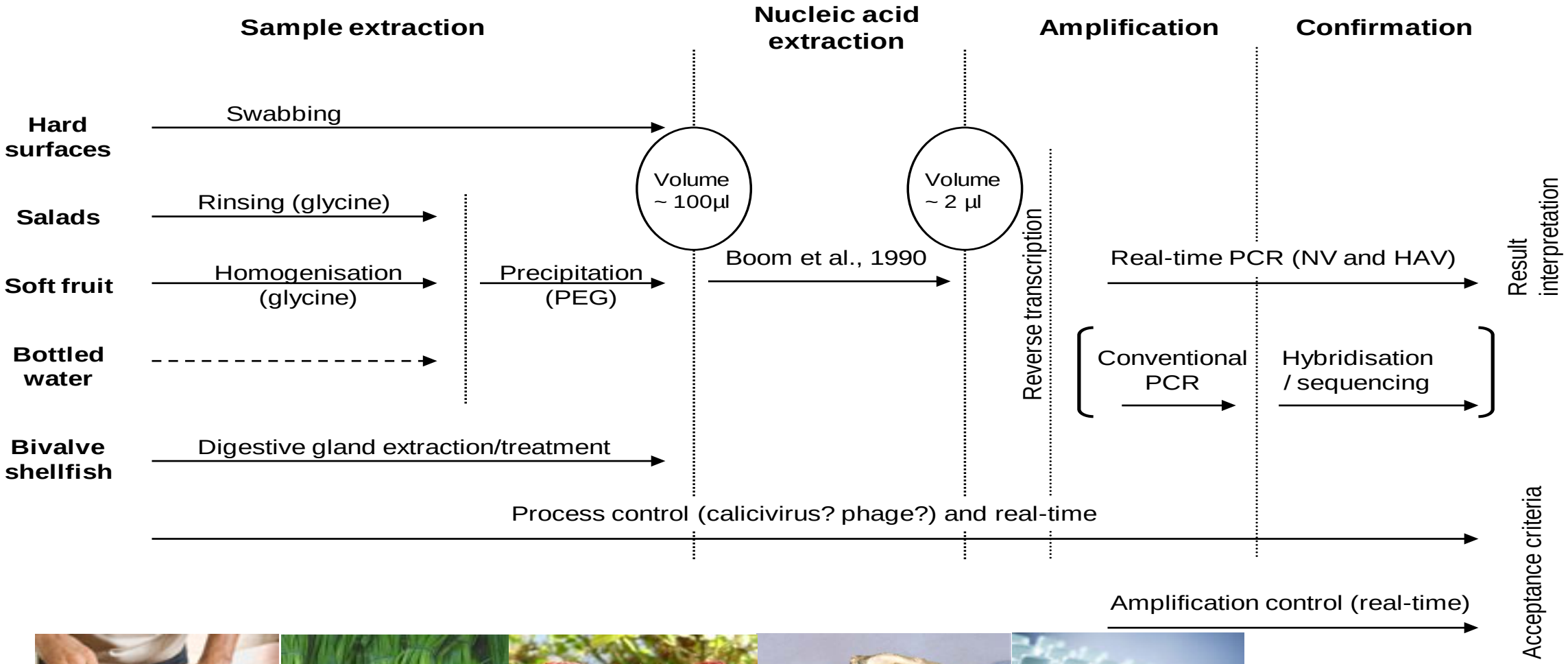
P I O N E E R I N G D I A G N O S T I C S

THE IDEAL METHOD FOR FOODBORNE VIRUSES DETECTION

- **Sensitive and specific**
- **Broadly reactive, detects all human genotypes**
- **Can be used for detection and genotyping**
- **Rapid or, better, real-time results**
- **Low detection limit**
- **Easy to use, portable and without requiring specialized equipment**
- **Works on a variety of sample types (food or environmental) and with adapted sampling protocols**
- **Able to distinguish between infectious and non-infectious virus**

THE REFERENCE METHODS: ISO15216-1 AND ISO15216-2

Target viruses: Norovirus, Hepatitis A virus







THE REFERENCE METHODS: ISO 15216-1 & 2

Mandatory quality controls ISO 15216

CONTROLS	ISO 15216-1 & 2
ANALYSES	REPRODUCTIBLE & REPEATABLE
COMPLEX METHOD	Several controls for each steps
EXTRACTION EFFICACY	VIRUS PROCESS CONTROL [MENGOVIRUS Vmc0]
RT-PCR EFFICIENCY	Internal positive control (RNA molecules)
QUANTIFICATION	PLASMIDS, dsDNA molecules
RT-PCR	CONTROLS [ARN VIRAL OU PLASMIDES]
NEGATIVE CONTROLS	PRETREATMENT & RT-PCR

THE REFERENCE METHODS: ISO15216-1 AND ISO15216-2

Pros

- Major viruses and food matrices included
- Simple set-up with detailed protocols on reagent and equipment
- Increases confidence on the results due to use of controls and details on how to interpret results
- International recognition of ISO method leading to increased implementation
- Enables the formulation of guidelines
- Possibility to compare and evaluate results from different labs (proficiency testing available)
- Facilities accreditation of laboratories for virus testing
- Some commercial solution based on these ISO are available

Cons

- Improvements of method may be slowed or halted
- Does not include methods for processed food matrices
- High number of controls increases costs
- Cannot distinguish between infectious and non-infectious particles
- Method complexity

Note: BAM method based on ultracentrifugation available for HAV in limited food matrices

Pros

- Uses in outbreak investigations and provide data for risk assessments
- Routine quantification provides data on baseline levels of viruses in food and will inform implementation of acceptable levels
- Systematic confirmation of RT-qPCR results by sequencing provides information on virus strain epidemiology

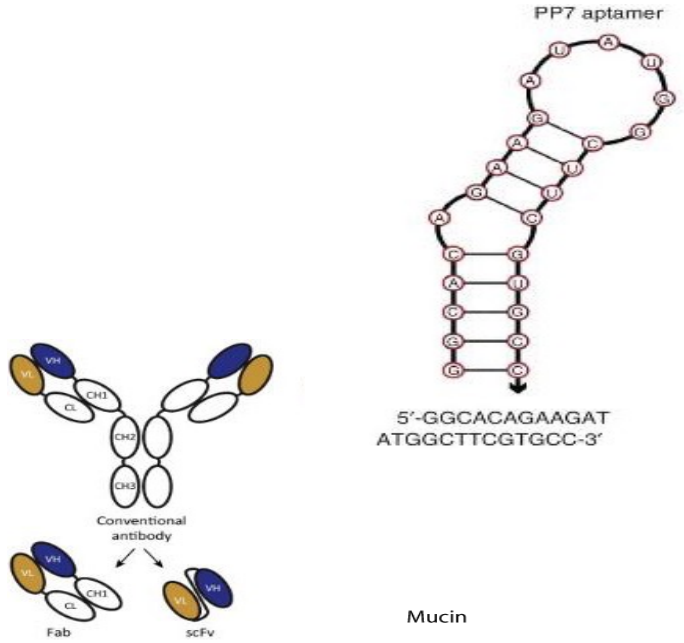
QUANTIFICATION AND CONFIRMATION

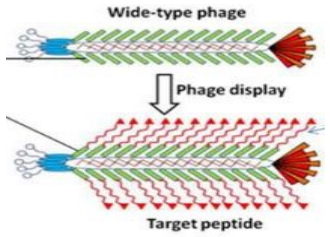
Cons

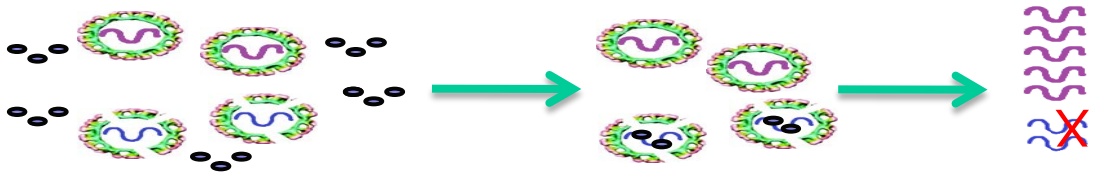
- Confirmation of RT-qPCR positive results by sequencing is difficult due to low sensitivity
- Viruses in foods are not evenly distributed
- Low levels of viruses can lead to variation of up to 1 log
- Short amplicons may not be suitable for typing
- Quantification and confirmation increase cost
- Time consuming

DETECTION FROM INTACT VIRUS CAPSIDS

- Use of RNase treatments
- Intercalating Dyes: Propidium or Ethidium Monoazide (PMA or EMA)
- Histo-blood group antigen (HGBA) glycans
- Monoclonal and polyclonal antibodies
- Nucleic acid aptamers and phage display
- Detection of oxidative damages on capsid proteins







DETECTION FROM INTACT VIRUS CAPSIDS

Pros

- Reduces overestimation of the number of infective virus particles

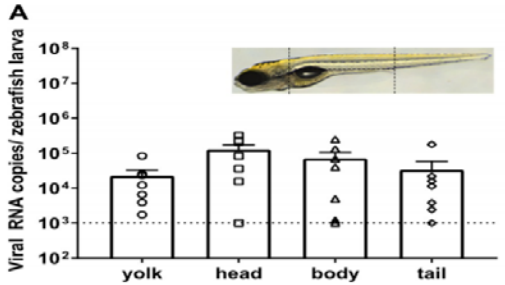
Cons

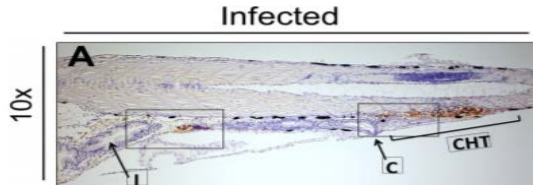
- A broad range of reagents need to be developed
- Needs careful evaluation of protocols according to type of matrices and different viruses and genotypes
- Infective and non infective controls must be included, no standardization
- Increased costs compared to standard ISO method

DETECTION OF INFECTED VIRUSES

Cell culture

- Available only for some strains of HAV, not easy to apply for routine detection in food samples
- Real breakthrough for NoV:
  - ✓ replication of human norovirus in cell stem-derived human enteroids (Ettayabi et al., 2016). Complex method that need to be optimized
  - ✓ Replication of norovirus in zebrafish larvae (Van Dycke et al., 2019), seems to be a simple replication method
- Not for routine testing in food
- Cost and time effective





- Mainly use for evaluation of the effectiveness of control strategies, inactivation methods (impact of cleaning process, evaluation of disinfectant, impact of food process…)

DETECTION OF INFECTED VIRUSES

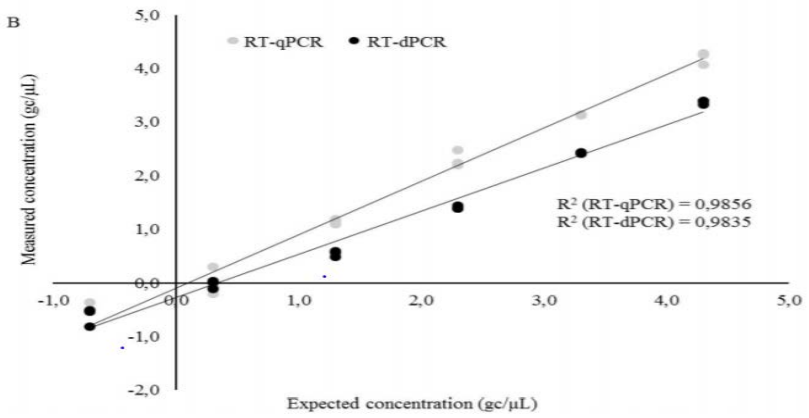
ICC-RTqPCR

- Integrated cell culture - RT-qPCR: cell culture prior molecular detection = increase of sensitivity
- Described for HAV not for NoV
- Detect viruses that do not show cytopathogenic effect
- Shorten time for analysis in comparison to cell culture
- High cost
- No standardization

NEW TECHNOLOGIES: DIGITAL PCR

Pros

- Reduces overestimation of the number of infective particles
- Improves detection sensitivity
- Improves accuracy



Cons

- Broad range of reagents need to be develop
- Needs careful evaluation of protocols according to type of virus and matrices
- Controls for infectious and non-infectious particles
- Increased costs compared to standard PCR method
- One-step format not available for digital PCR

NEW TECHNOLOGIES: NEXT GENERATION SEQUENCING

Pros

- Viral/virome identification
- Provide data to improve PCR assays
- Improve knowledge on bacterial/viral contamination (Strubbia et al., 2019, Front Microbiol: NoV diversity in sewage and oysters)
- Could be used for food analysis in the future

Cons

- Increase cost and time for sample prep
- no standardized protocols

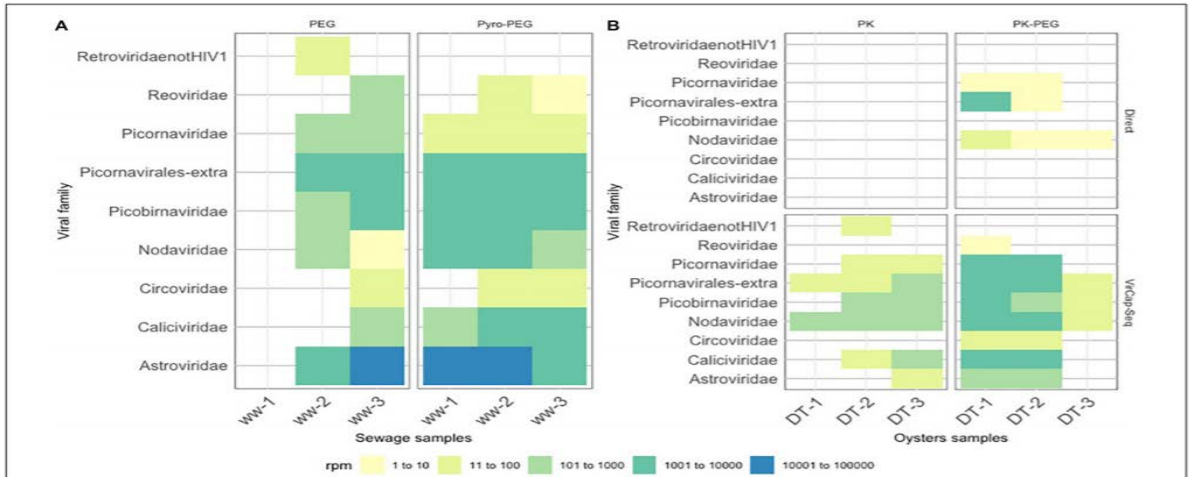


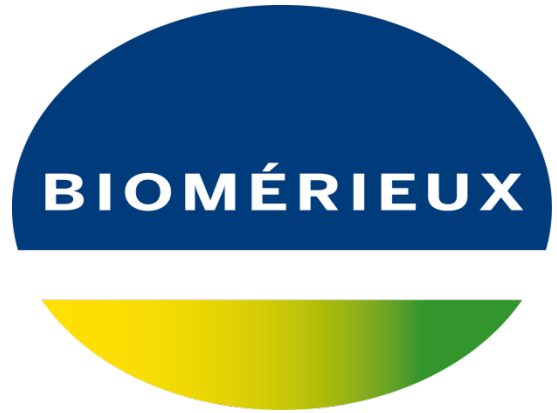
FIGURE 6 | Heat-map representing virus diversity and the number of reads in each sample. Reported contigs with a sequence length ≥500 bp and identity score ≥85% were mapped toward the contigs obtained with SLIM. Values were grouped into five categories and depicted with the following colors: light yellow for values between ≥1 and ≤10 reads, lime between ≥11 and ≤100, light green between ≥101 and ≤1000, dark green between ≥1001 and ≤10000 and blue between ≥10001 and ≤100000 reads. **(A)** displays the results of the sewage samples (ww) used in the three bioaccumulation experiments, while **(B)** displays the results of the oyster digestive tissues (DT).

PROS AND CONS OF EXISTING METHOD

Method	Advantages (pros)	Disadvantages (cons)
ISO/CEN method	• Major viruses and food matrices are included • Increased confidence in the results due to use of controls and detailed description of how to interpret results; • International recognition of an ISO method increases implementation of a harmonized method in laboratories; • Introduces the possibility to compare and evaluate results from different laboratories; • Facilitates accreditation of laboratories for virus testing.	• Improvements of the methods may be halted • Does not include methods for processed food matrices; • The high number of controls increases costs; • Commercial controls must be available; • May lead to non-detection of low levels of virus in some specific matrices; • Cannot distinguish between infectious and non-infectious particles; • Method complexity.
Quantification and confirmation	• Routine quantification provides data on baseline levels of viruses in food matrices and will inform implementation of acceptable levels; • Systematic confirmation of RT-qPCR results by sequencing provides information on virus strain epidemiology	• Quantification by RT-qPCR is sensitive to inhibitors and can be unreliable as accuracy for low levels of virus; • Confirmation of RT-qPCR positive results by sequencing is difficult due to low sensitivity; • Quantification and confirmation increase cost; • Time consuming.
Molecular virus detection for intact virus capsids	• Reduced overestimation of the number of infective virus particles.	• A broad range of reagents needs to be developed; • Needs careful evaluation of protocols according to type of virus and matrices; • Infective and non-infective controls must be included; • Increases costs compared to standard PCR method.
Detection of infective viruses	• Allows detection of infectious viruses • ICC-RT-PCR ○ Is more sensitive than cell culture alone; ○ Detects infectious viruses that do not show cytopathogenic effect; ○ Shortens the time for analysis compared to cell culture alone	• Wild-type enteric viruses are generally difficult to cultivate; • A simple cultivation system for NoVs need to be optimzed; • Cultivation increases the cost and time needed for diagnostics; • ICC-RT-PCR is not quantitative unless used as a Most Probable Number (MPN) test.
New technologies	• Digital PCR ○ Is less sensitive to inhibitors in food matrices; ○ Provides more accurate quantification independent of standard curves; • Next generation sequencing can pick up emerging viruses and new virus strains.	• Increased costs and sample preparation; • Absence of standardized approach for next generation sequencing.

There is still a lot of work to do for a simple routine method

Bosch et al., Int J Food Microbiol, 2019



PIONEERING DIAGNOSTICS

Risk Analysis Framework

Risk assessment: Assessing the probability and severity of an adverse health effect consequential to a hazard present in food.

Risk management: Selecting, implementing and monitoring suitable options to accept, minimize or reduce the assessed risk after carefully evaluating the contents of the risk assessment

Risk communication: interactive information and opinion exchange between risk assessors, risk managers, consumers, food businesses, academics and other interested parties.

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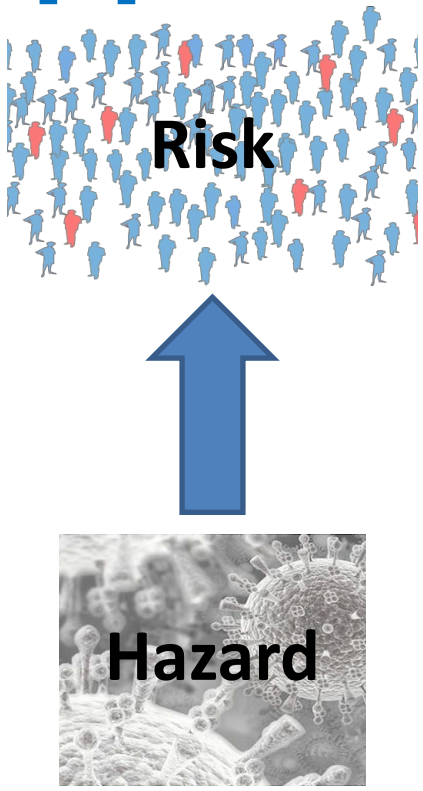
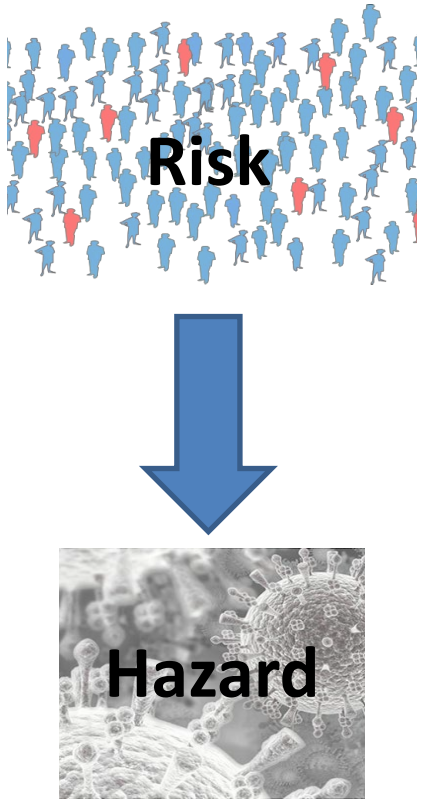
Risk Assessment Approaches



Bottom-up risk assessment
(food chain-based)



Top-down risk assessment
(epidemiology-based, surveillance-based)

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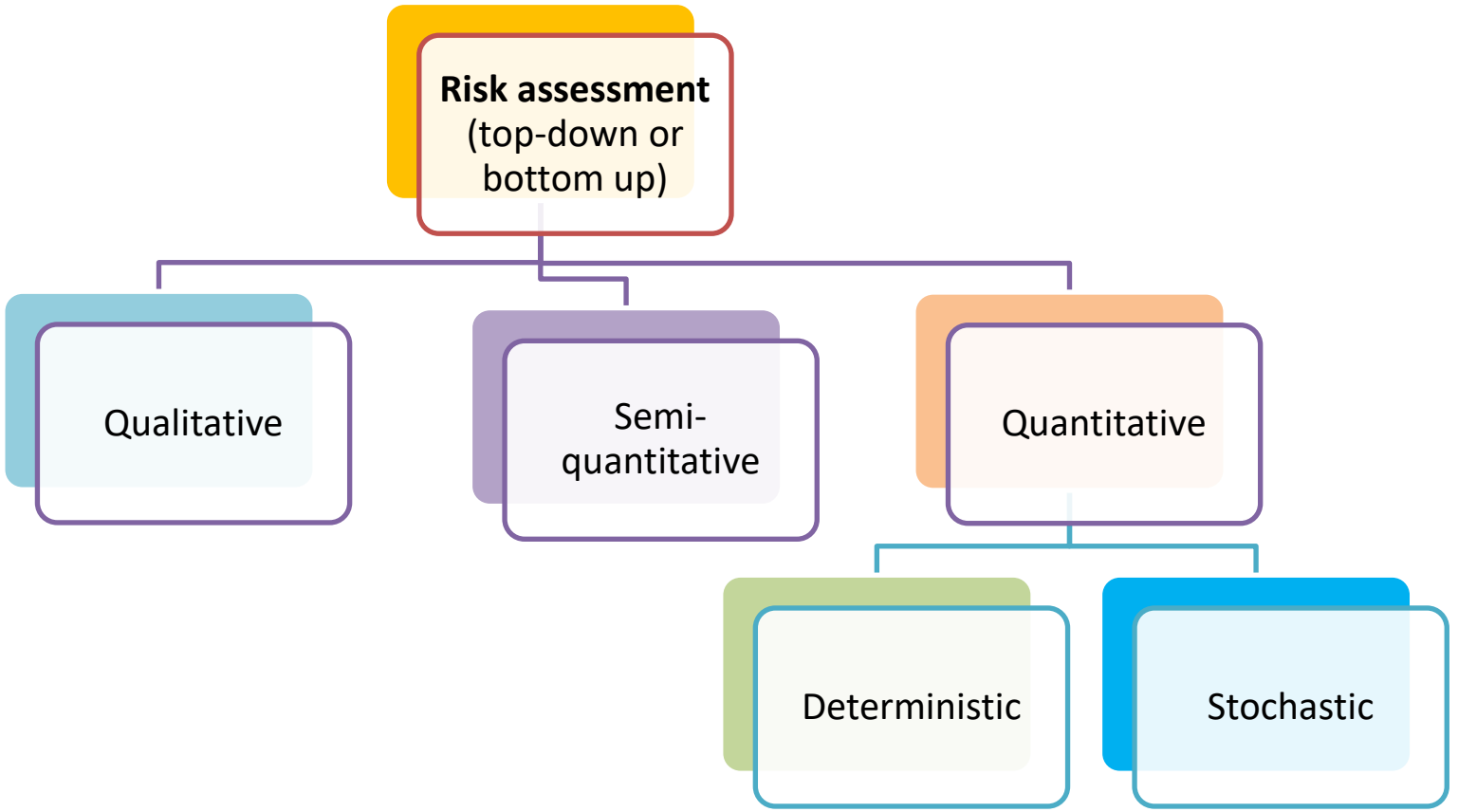
Risk Assessment Types



Bottom-up Risk Assessment

Hazard identification

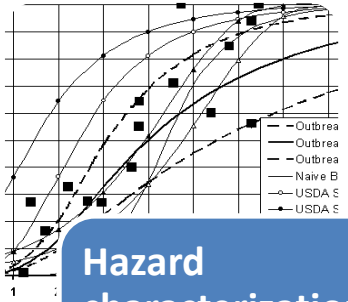
- Which hazards in food have the potential to cause an adverse health effect?

- Mode of production?
- Routes of contamination?
- Product formulation?
- Product association with specific hazards?

Exposure assessment

- What is the intake of the hazard through food and if relevant from other sources?

- Initial concentration?
- Prevalence?
- Hazard increases, decreases, or remains stable?
- Cross-contamination?



Hazard characterization

- What is the response to the hazard for different potential doses through food?

- Dose response curve (epidemiological data)
- Healthy vs susceptible population?
- Portion sizes?

Risk characterization

- What is the probability and severity of the effect in relation to this hazard in food?

- Frequency of consumption ?
- Population immunity?

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Top-down Risk Assessment

Reported risk

- What is the reported incidence of illness due to this hazard?

National surveillance system:

- Epidemiological data (outbreaks, notification data)

Population risk

- What is the actual incidence of illness in the community?

Active surveillance:

- Underreporting rate

Foodborne risk

- What is the incidence due to food?

Source attribution:

- Food
- Environment
- Travel
- Human
- Animal

Foodborne hazard

- Priorities in terms of products /product groups for managing the hazard?

- Food product or product group source attribution
- Risk ranking

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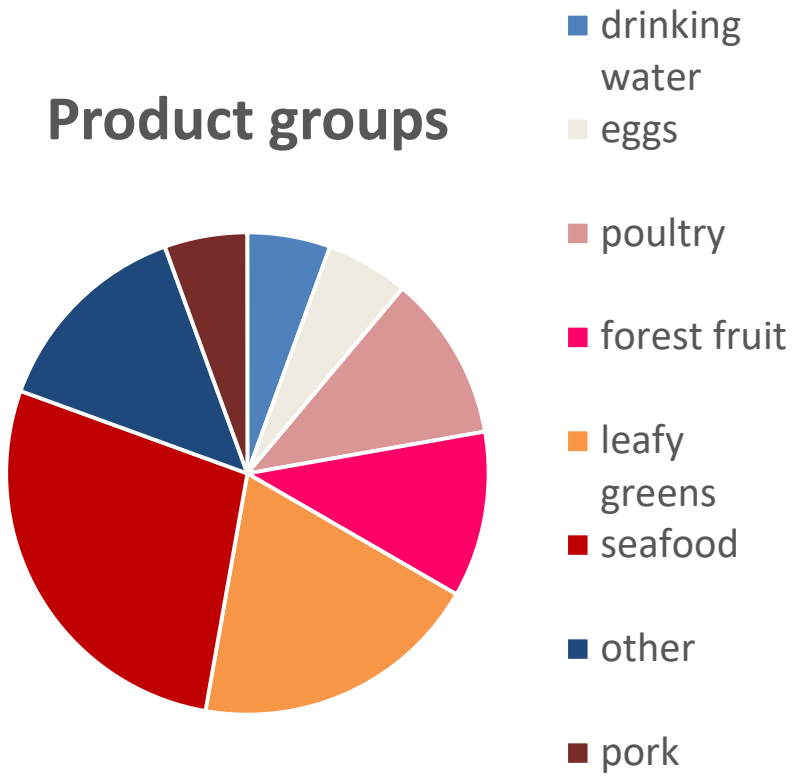
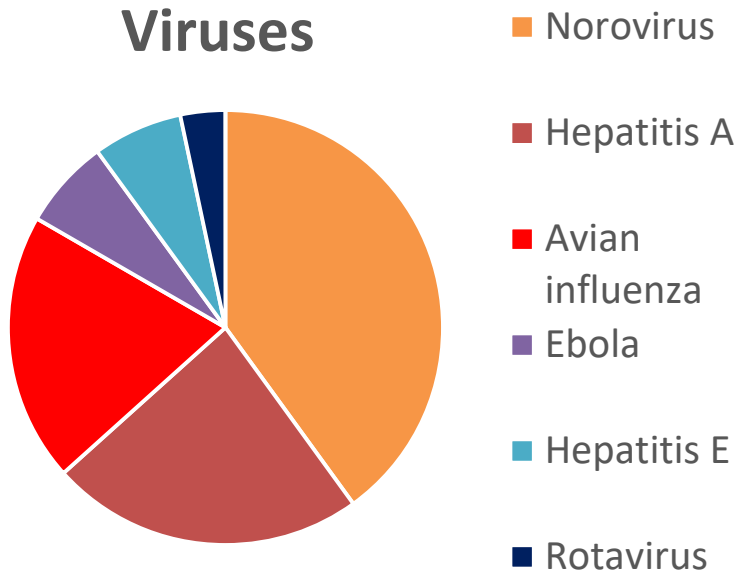
Overview of Bottom-up Risk Assessments

- 23 publications
- 36 product-virus combinations
- 6 viruses, 8 product groups
- 3/23 qualitative, 3/23 deterministic, 17 quantitative





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Top-Down vs Bottom-Up Risk Assessments

Bottom-up risk assessments	Top-down risk assessments
• Interventions • Risk for standard industry practices • More focus on interventions than on risk • Industry/food chain safety management	• Interventions • Risk for incidental contamination events • More focus on risk than on interventions • Public health authorities/governmental food safety management

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Most Important Interventions for the Control of Viruses

- Setting adequate targets for inactivation
  - e.g. 85-90ºC for at least 1.5 min (CAC, 2012)
- Raw material/food production controls
  - GAP, GHP, GMP + validation & verification
- Increased surveillance of high risk food commodities
  - e.g. soft fruits (European Commission, 2012)
- Control spread via food handlers
  - e.g. adequate hand hygiene + suitable period of absence/sickness leave

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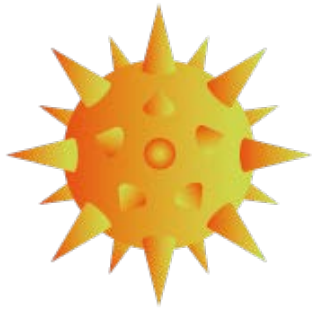
Effect of Processing Technologies to Control Viruses in Foods



Sophie Zuber, PhD
Nestlé Research, Switzerland

Member of the ILSI Expert Group on Control options for Viruses in Food Processing

Webinar, November 12, 2019

Outline

Set the scene

- Recent virus outbreaks - Critical raw materials – Surveillance data
  - ➢ Which matrix-process combinations need validation?

Virus inactivation studies

- Challenges for validation
  - ➢ Examples: Thermal processing, HPP, gaseous ozone
  - ➢ Outlook

Recent RASSF virus alerts and outbreaks

10/07/2019	2019.2492	Spain	norovirus (GI and GII /2g) in live venus clams (Chamelea gallina) from Italy
05/07/2019	2019.2407	Spain	norovirus (genogroup II) in live oysters from France
	2019.2415	Spain	norovirus (GI /2g) in live oysters (Crassostrea gigas) from France
	2019.2374	Spain	norovirus (GI, GII /2g) in live venus clams (Chamelea gallina) from Italy

Public Health Alert Concerning Hepatitis A Virus Contamination of Kroger Brand Frozen Blackberries and Costco Kirkland Signature Brand Three Berry Blend

June 2019

Dates from Iran linked to Hepatitis A outbreak for second time in 2 years

By Joe Whitworth on May 1, 2019

AEM Journals.ASM.org

Surveillance Study of Hepatitis A Virus RNA on Fig and Date Samples

Ingeborg L. A. Boxman, Nathalie A. J. M. te Loeke, Kyara Klunder, Geke Hägele, and Claudia C. C. Jansen
Food and Consumer Product Safety Authority, Zutphen, The Netherlands

A total of 91 fig and 185 date samples were analyzed by reverse transcription (RT) real-time PCR for the presence of hepatitis A virus (HAV) RNA. Two batches of dates tested positive, and the HAV RNA detected was genotyped as IA. These findings warrant further development of methods applicable to food which is consumed untreated and is exported from countries in which HAV is endemic.

Effectiveness of control measures: Target reduction level for viruses?

$$H_0 - \Sigma R_{A,B,C} + \Sigma I_{A,B,C} \leq POs$$ [1]

Initial load at primary production

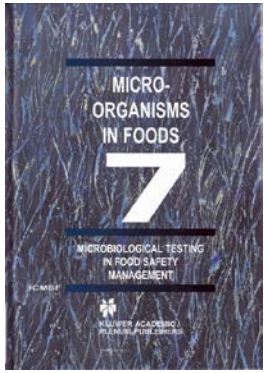
Reduction (Supplier and Factory)

Increase (Growth, Recontamination)

Performance objective

- Prevalence is based on detection by qPCR

- For NoV only surrogate inactivation data available
- Validation data from lab scale studies only



[1] ICMSF conceptual equation

NoroCORE
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NOROVIRUS, CULTURED.

A 48 YEAR MYSTERY SOLVED

Dr. Mary Estes and her Lab at Baylor College of Medicine have successfully cultured human norovirus in intestinal cells.

Scientists have been trying to culture the virus since the first norovirus outbreak was described in 1968.

The lack of an *in vitro* culture system has long been considered the single greatest barrier to norovirus research.

HISTORY OF NOROVIRUS RESEARCH

1929
RUMORED
Dr. John Zahorsky, a pediatrician, gives the name "winter vomiting disease" to a common childhood illness that causes vomiting, diarrhea, and a fever.

1968
DESCRIBED
An elementary school in Norwalk, OH experiences an outbreak of "winter vomiting disease." A virus is suspected.

1972
VISUALIZED
The Norwalk virus is first seen by Dr. Albert Kapikian and his team at NIH using immune electron microscopy (IEM).

1990
CLONED
The Norwalk virus genome is cloned, paving the way for an era of molecular studies.

1992
CREATED
Empty shells of norovirus proteins (capsids) are artifically created by the Estes Lab. These virus-like particles are not infectious and enable studies of the capsid.

2016
CULTURED
Human noroviruses are successfully cultured by Dr. Mary Estes and her team at Baylor College of Medicine.

READ THE ARTICLE

K. Ettayebi *et al.*, *Science* 10.1126/science.aaf5211 (2016).

WHAT IS NOROVIRUS?

- It is a tiny (≈27nm), spherical virus belonging to the *Caliciviridae* family.
- It is the most common cause of diarrhea in the world and the most common cause of foodborne illness in the United States.
- An estimated 1 in 15 Americans experience the virus each year, amounting to around 20 million cases.

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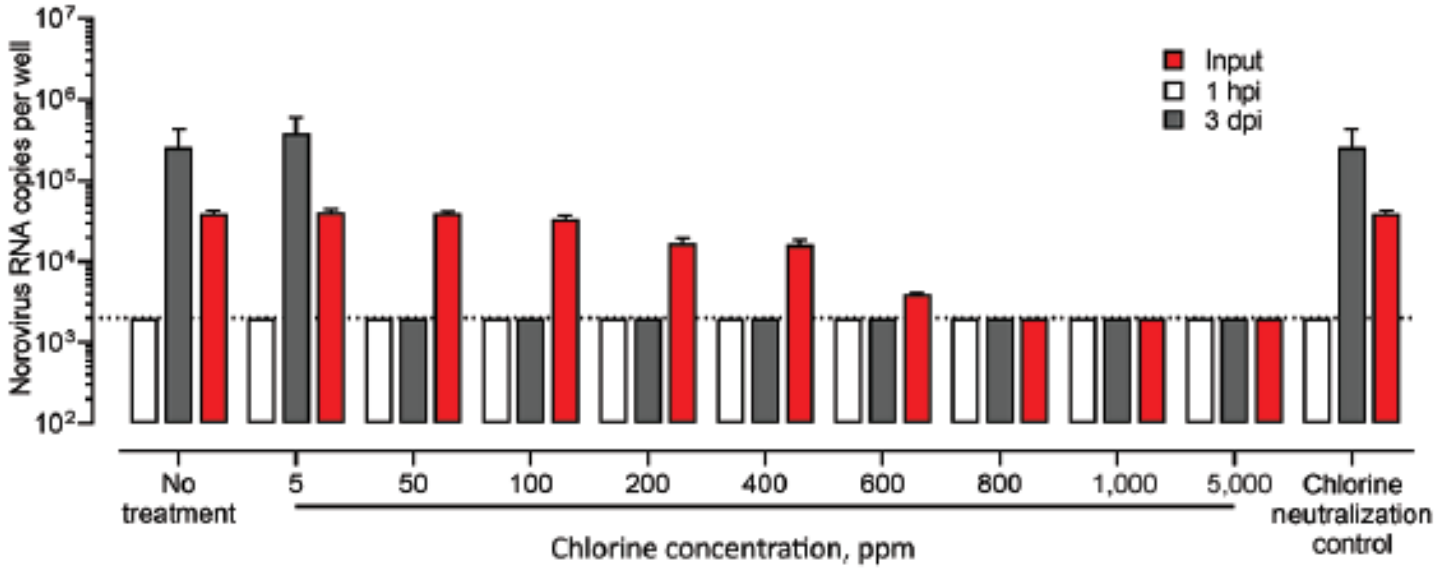
Human Norovirus Replication in Human Intestinal Enteroids as Model to Evaluate Virus Inactivation

Veronica Costantini, Esther K. Morantz, Hannah Browne, Khalil Ettayebi,
Xi-Lei Zeng, Robert L. Atmar, Mary K. Estes, Jan Vinjé



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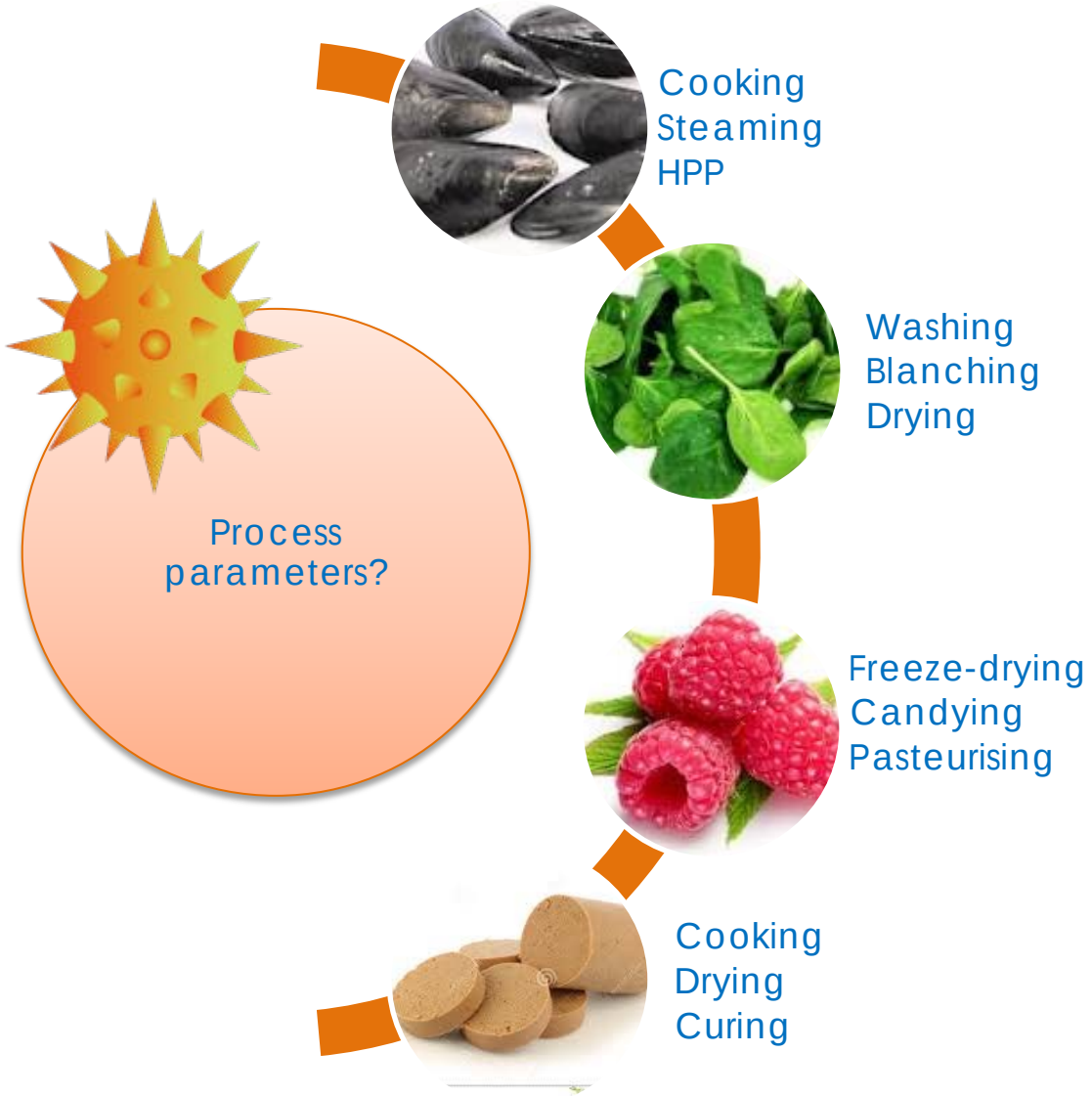
Which matrix-process combinations?



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Review

Foodborne viruses: Detection, risk assessment, and control options in food processing

Albert Bosch[a], Elissavet Gkogka[b], Françoise S. Le Guyader[c], Fabienne Loisy-Hamon[d], Alvin Lee[e], Lilou van Lieshout[f,*], Balkumar Marthi[g,h], Mette Myrmel[i], Annette Sansom[j], Anna Charlotte Schultz[k], Anett Winkler[l], Sophie Zuber[m], Trevor Phister[n]

- Chilled & frozen storage
- pH, a_w
- Antiviral food component & packaging
- Sanitizers
- Thermal processing
- High pressure processing
- Irradiation

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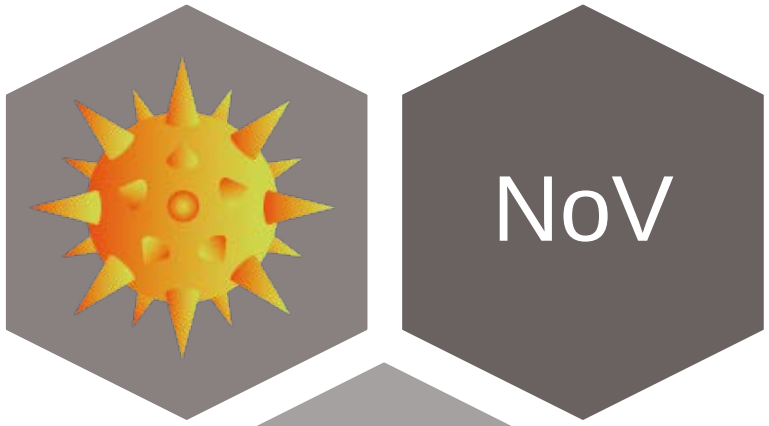
Virus inactivation studies: Challenges



NoV

HAV

HEV

Pathogen versus surrogate

- MNV (Murine Norovirus)
- FCV (Feline calicivirus)
- TV (Tulane virus)

- HAV HM-175

- HEV genotype 3 strain 47832c

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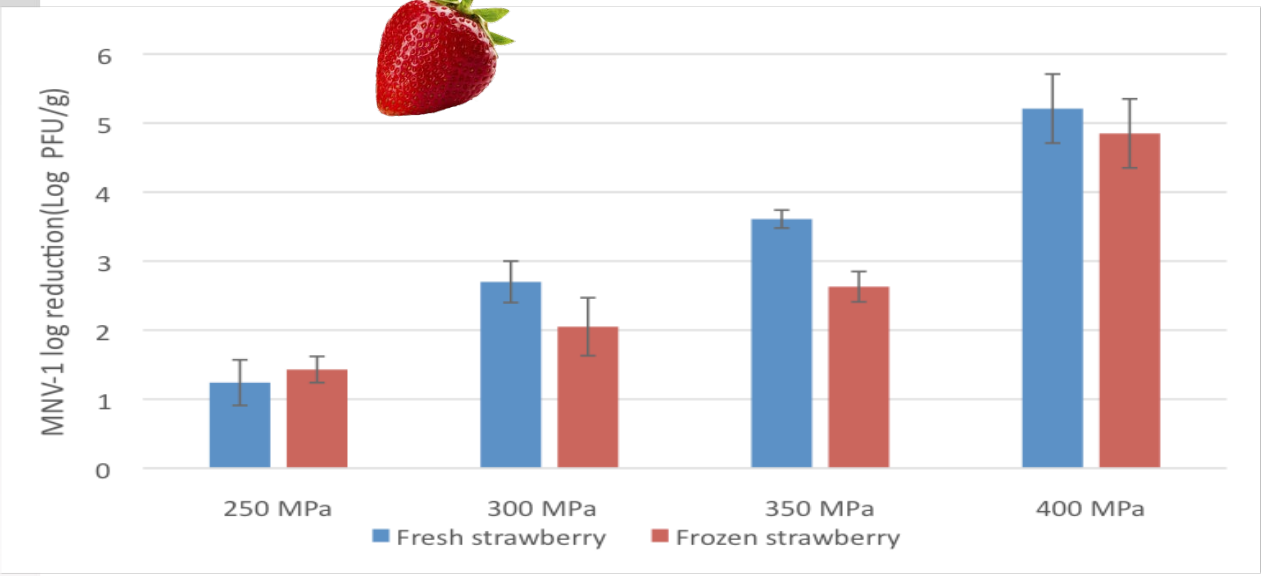
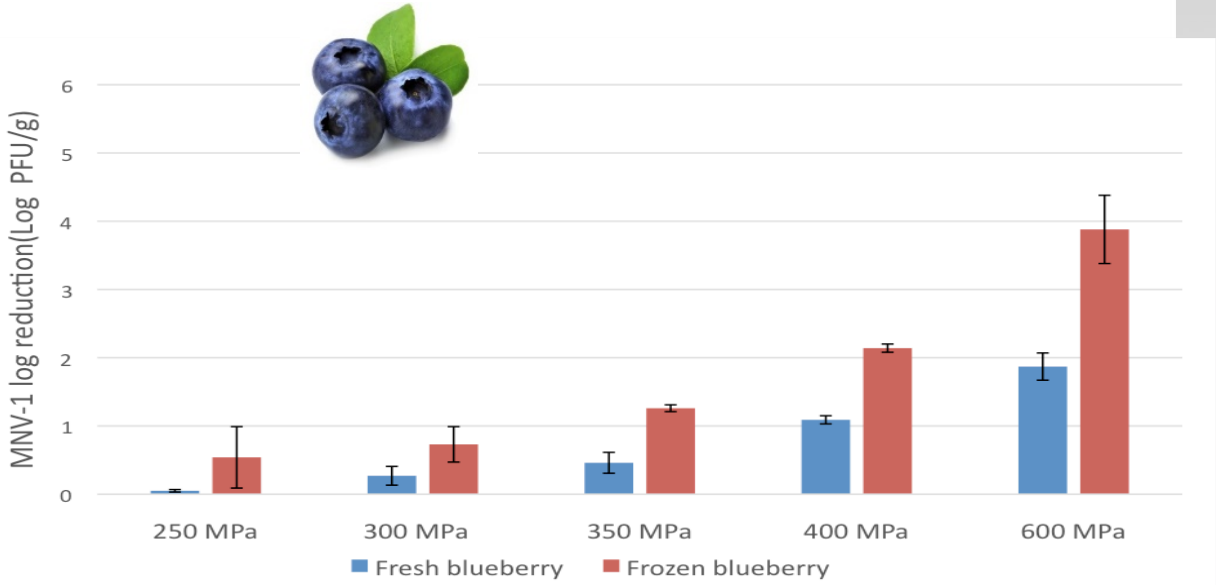
NoV and its surrogates: Thermal processing

Control measures	Matrix	Virus	Log_{10} reduction	Reference
72°C, 1 min	Water	MNV	>3.5	Hewitt *et al.*, 2009
80°C, 1 min	Spinach	MNV	≥ 2.4	Baert *et al.*, 2008
75°C, 0.25 min	Raspberry puree	MNV	2.8	Baert *et al.*, 2008
95°C, 2.5 min	Basil	FCV	> 4	Butot *et al.*, 2009
60°C, 15 min	Stool	HuNoV	>5	Ettayebi *et al.*, 2016

➢ How will HuNoV inactivation data compare with the different surrogates?

Application of HPP on fresh and frozen berries to inactivate Murine Norovirus: Matrix effect





➢ Higher inactivation of MNV on strawberries compared to blueberries

Ozone gas

& UNIVERSITEIT GENT

frontiers in Sustainable Food Systems

Inactivation of Foodborne Pathogens and Their Surrogates on Fresh and Frozen Strawberries Using Gaseous Ozone

Zijin Zhou[1], Sophie Zuber[2], Frédérique Cantergiani[2], Imca Sampers[3], Frank Devlieghere[1] and Mieke Uyttendaele[1]*

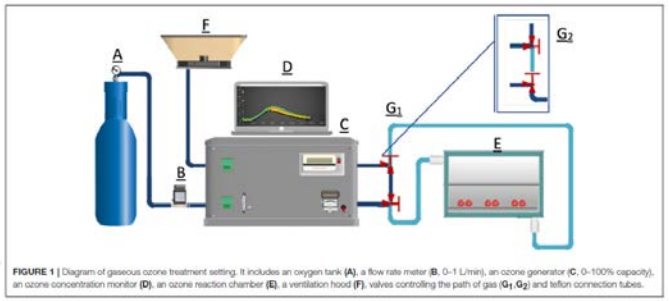
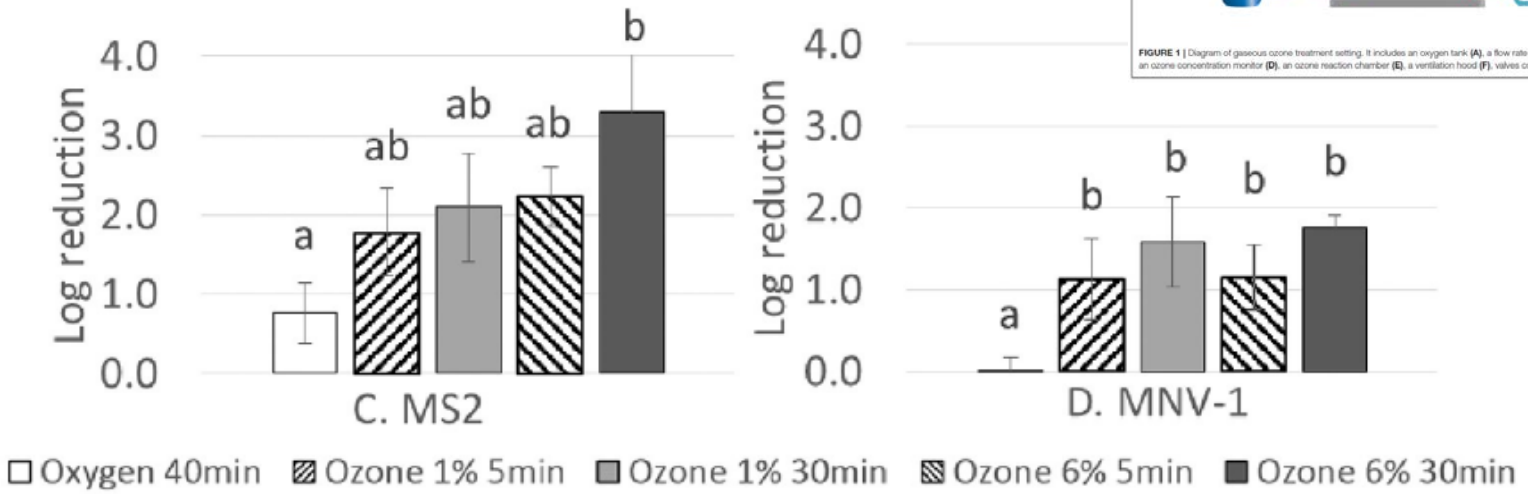


FIGURE 1 | Diagram of gaseous ozone treatment setting. It includes an oxygen tank (A), a flow rate meter (B, 0–1 L/min), an ozone generator (C, 0–100% capacity), an ozone concentration monitor (D), an ozone reaction chamber (E), a ventilation hood (F), valves controlling the path of gas (G_1, G_2) and teflon connection tubes.



- At 6% ozone for 30 min, 3.3 and 1,8 $\log_{10}$ for MS2 and MNV, respectively
- Pilot-scale trials of interest to the industry, but no suitable surrogate identified

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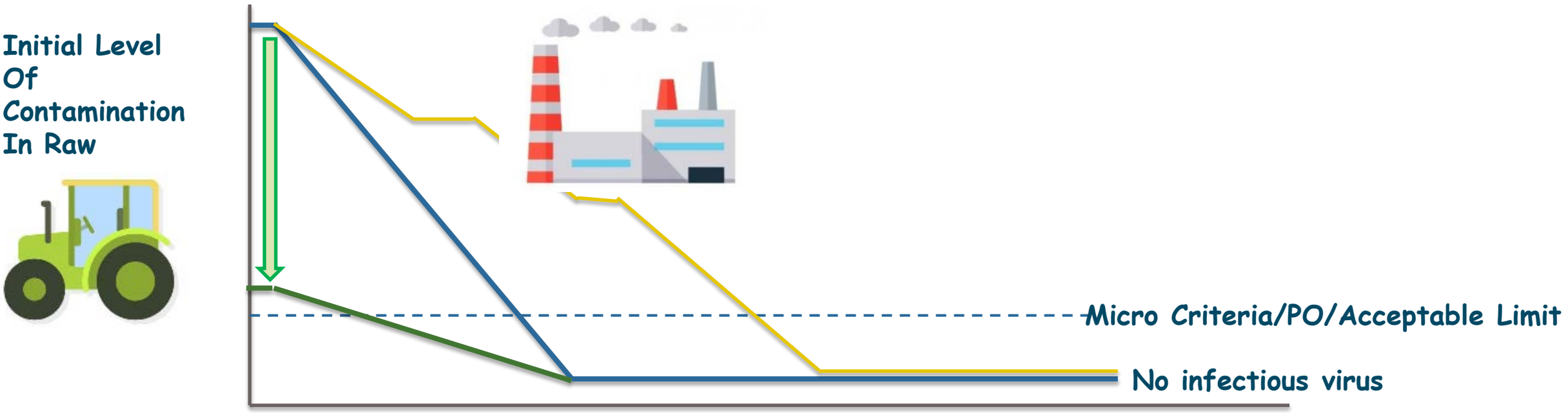
Processing options and their efficacy to reduce the virus risk

Highlights of using surrogates in processing technologies.	
Thermal processing	High inactivation of most surrogates at 75 °C in high water activity foods
High pressure processing	High inactivation of most surrogates between 400 and 600 MPa, except Poliovirus and Aichi virus
Frozen and chilled storage	Low reduction of most surrogates
pH and water activity	Low reduction of most surrogates, except FCV which is pH sensitive
Antiviral components and essential oils	Viral inactivation is time and concentration dependent
Sanitizers	Low inactivation of most surrogates on fresh produce
Light based technologies	High inactivation in clear liquids & on surfaces of most surrogates
Ionising radiation	Low reduction of most surrogates at FDA approved dosages

It is key to minimize the viral load in the field



… and to continue filling research gaps

- Work on wider application of cultivable HuNoV and HEV
- Develop surrogates for pilot-scale validations
- Fill gaps regarding surrogate choice, inoculum level and inoculation methods

Questions?

Questions should be submitted to the presenters during the presentation via the **Questions section** at the right of the screen.

Slides and a recording of this webinar will be available for access by IAFP members at www.foodprotection.org within one week.

International Association for Food Protection