Methods for detection of enteric viruses in food

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Monitoring Bacteriological and Viral
Contamination of Bivalve Molluscs

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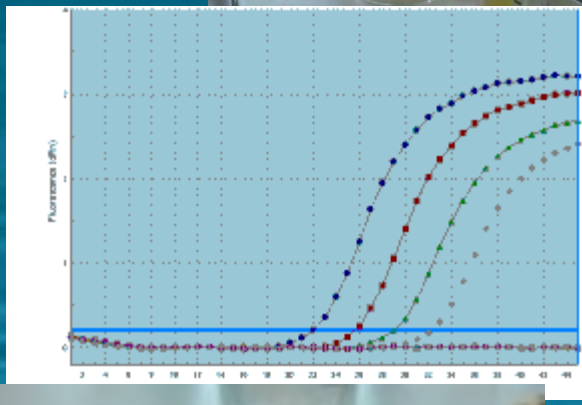
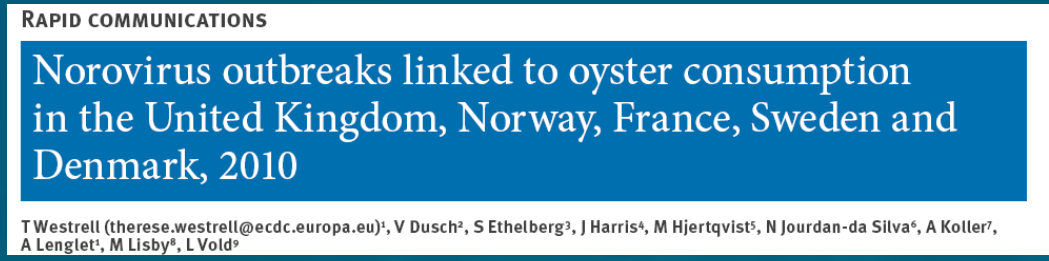
European Community Reference Laboratories Regulation (EC) No 882/2004, Article 32

- Coordination of and assistance to NRLs
- Analytical methods for Official Control testing
- Comparative (proficiency) testing
- New analytical methods (R&D)
- Training
- Advice to DG SANCO
- Collaboration with third countries



Background

- Virus outbreaks continue to occur in the EU and Internationally

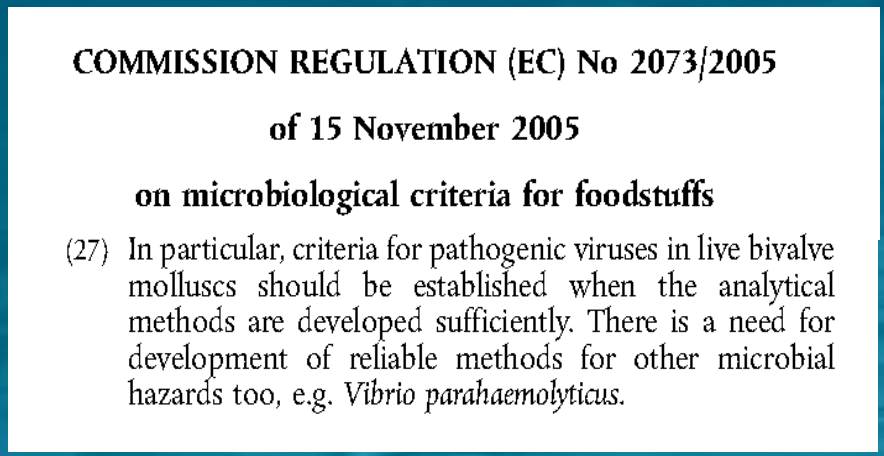


RAPID COMMUNICATIONS

Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010

T Westrell (therese.westrell@ecdc.europa.eu)[1], V Dusch[2], S Ethelberg[3], J Harris[4], M Hjertqvist[5], N Jourdan-da Silva[6], A Koller[7], A Lenglet[1], M Lisby[8], L Vold[9]

- European legislation foresees virus controls when the methods are sufficiently developed and available for use



COMMISSION REGULATION (EC) No 2073/2005

of 15 November 2005

on microbiological criteria for foodstuffs

(27) In particular, criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently. There is a need for development of reliable methods for other microbial hazards too, e.g. *Vibrio parahaemolyticus*.

- EURL responsible for analytical methods used in Official Controls

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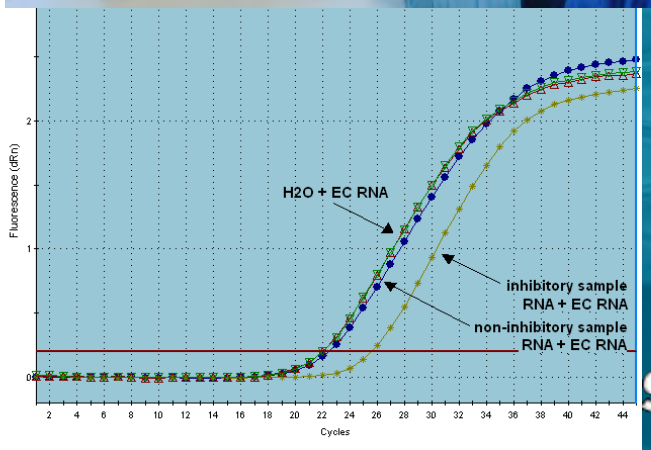
PCR methods reviewed in 2006

- 23 international labs involved in 2006 ring trial organised by EURL - detection of norovirus and HAV in contaminated oysters
- Virus extraction; 13 methods
- Viral RNA extraction; 29 methods
- RT-PCR; one and two-step, conventional single round, nested and semi-nested and real-time RT-PCR formats used
- Primers/probes; at least 13 different sets
- Development of standardised methodology necessary for harmonisation and consumer safety

ISO/CEN method

- EURL has lead method standardisation and validation for norovirus and hepatitis A in food
  - Chairs CEN/TC 275/WG6/ TAG4
  - 10 year development programme
  - Circa 50 participants from 13 countries
  - First ever ISO technical specification for viruses in food (ISO 15216 parts 1 and2) published May 2013
  - Standard protocols on EURL website
  - Formal multi lab validation of the virus method now completed



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

Framework for method

- Horizontal method (all foodstuffs included)
- Viruses of primary focus:
  - Norovirus
  - Hepatitis A virus
- Matrices of primary focus:
  - Food surfaces
  - Salad crops
  - Soft fruits
  - Bivalve shellfish
  - Bottled water





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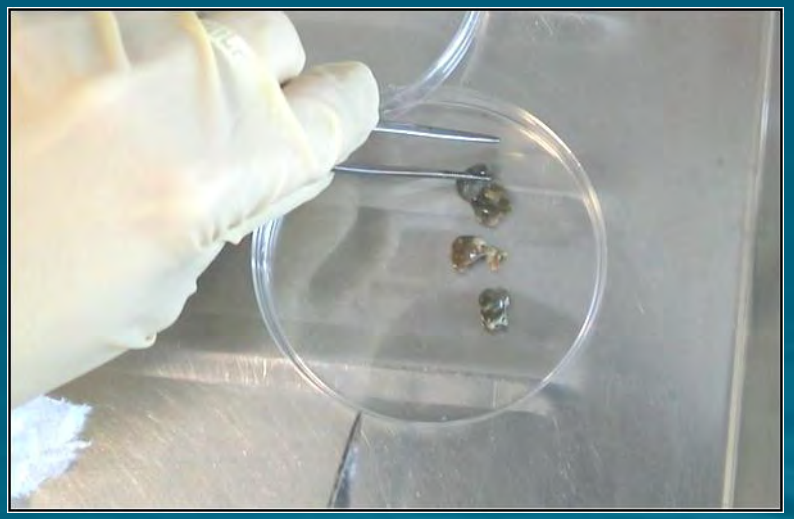
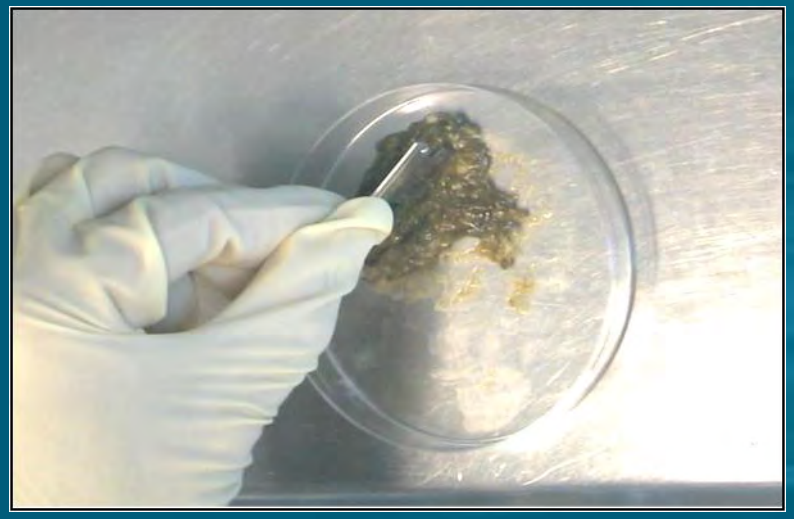
Digestive gland dissection





- Proteinase K digestion of chopped glands

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RNA extraction

- Boom technology (virus capsid disruption with chaotropic reagents, adsorption of RNA to silica particles)
- Use of magnetic silica technology preferred by many group members to centrifugation based protocol

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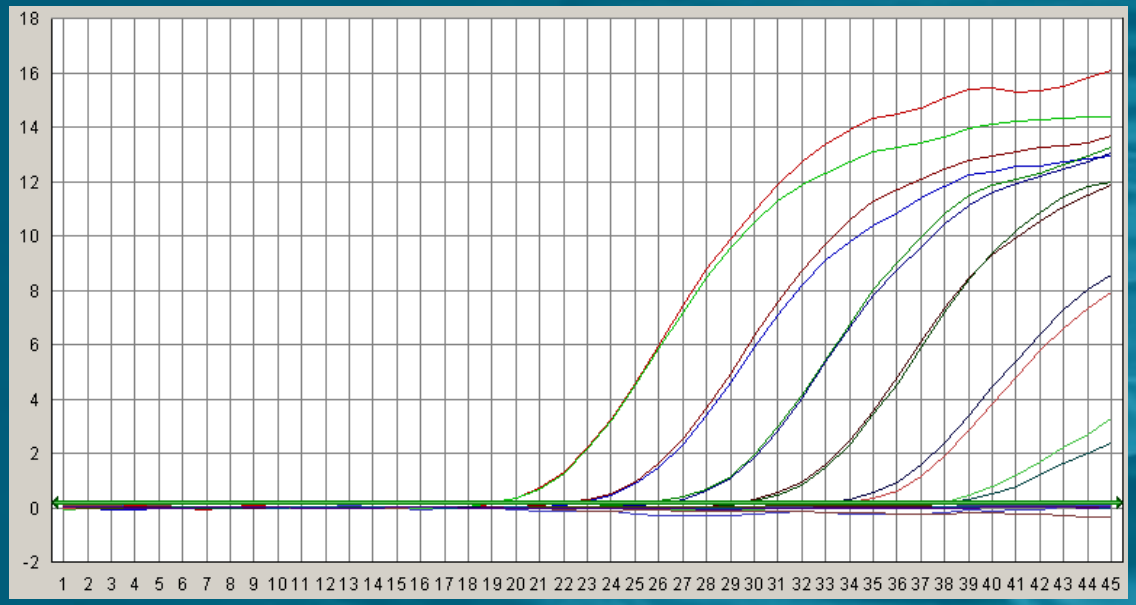
RT-PCR

- One-step TaqMan (“hydrolysis probe”) RT-PCR for all targets
- Standard stipulates that primers and probes “must be published in a peer-reviewed journal and be verified for use against a broad range of strains of target virus”
- Norovirus primers must target junction of ORF1/2
- HAV primers must target 5’ NCR



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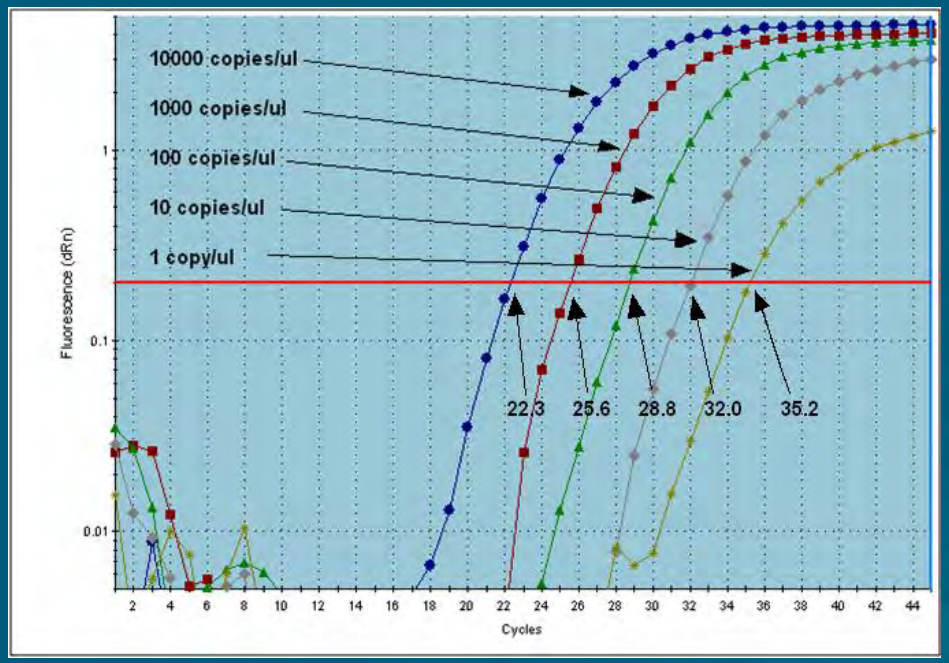
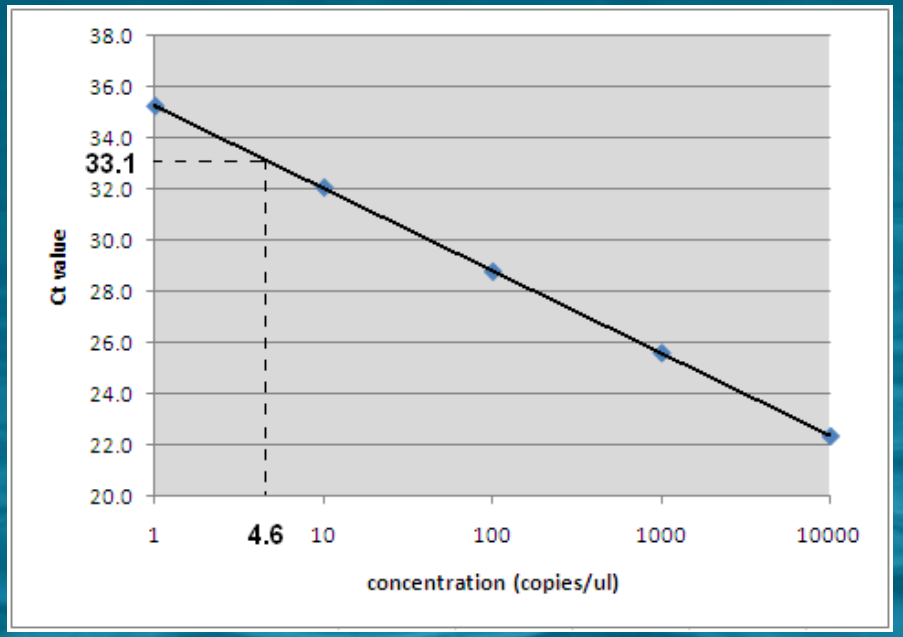
Quantitation using standard curve





- Reporting in genome copies per gram of matrix tested

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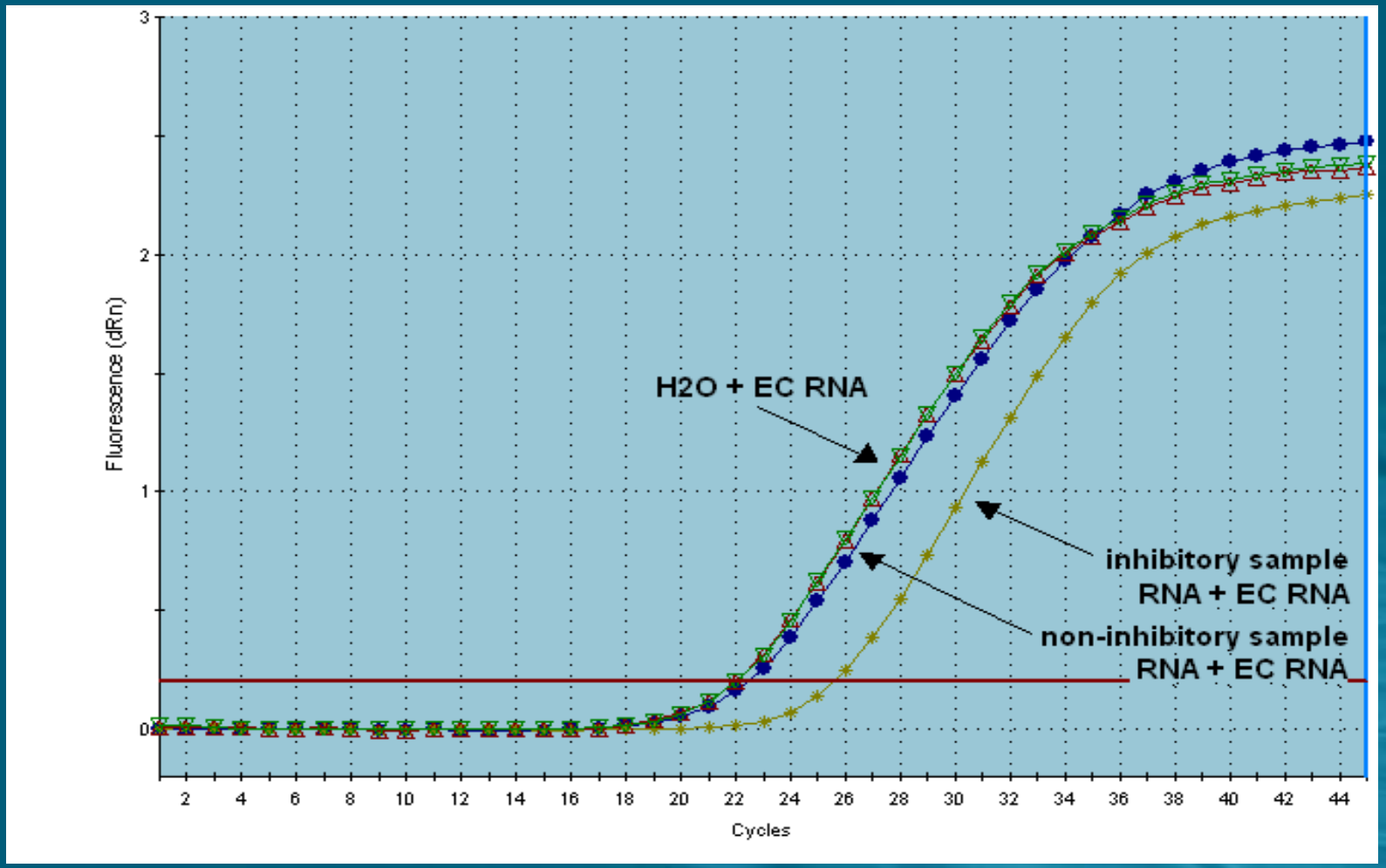
QC criteria



- Set QC criteria for: inhibition and recovery

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TECHNICAL SPECIFICATION

ISO/TS 15216-1

First edition

Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR —

Part 1: Method for quantification

Microbiologie des aliments — Méthode horizontale pour la recherche des virus de l'hépatite A et norovirus dans les aliments par la technique RT-PCR en temps réel —

Partie 1: Méthode de quantification

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Validation of ISO/TS 15216

- Maximum lifespan of technical specification 6 years; requires validation to convert to “full” standard
- European project currently underway to validate TS 15216-1 (quantification) in 7 food matrices
  - Oysters
  - Mussels
  - Raspberries
  - Lettuce
  - Spring Onions
  - Bottled Water
  - Food Surfaces (Bell Pepper)
- In two stages
  - Method characterisation in single labs
  - Inter laboratory trials
- Generation of data complete; analysis ongoing

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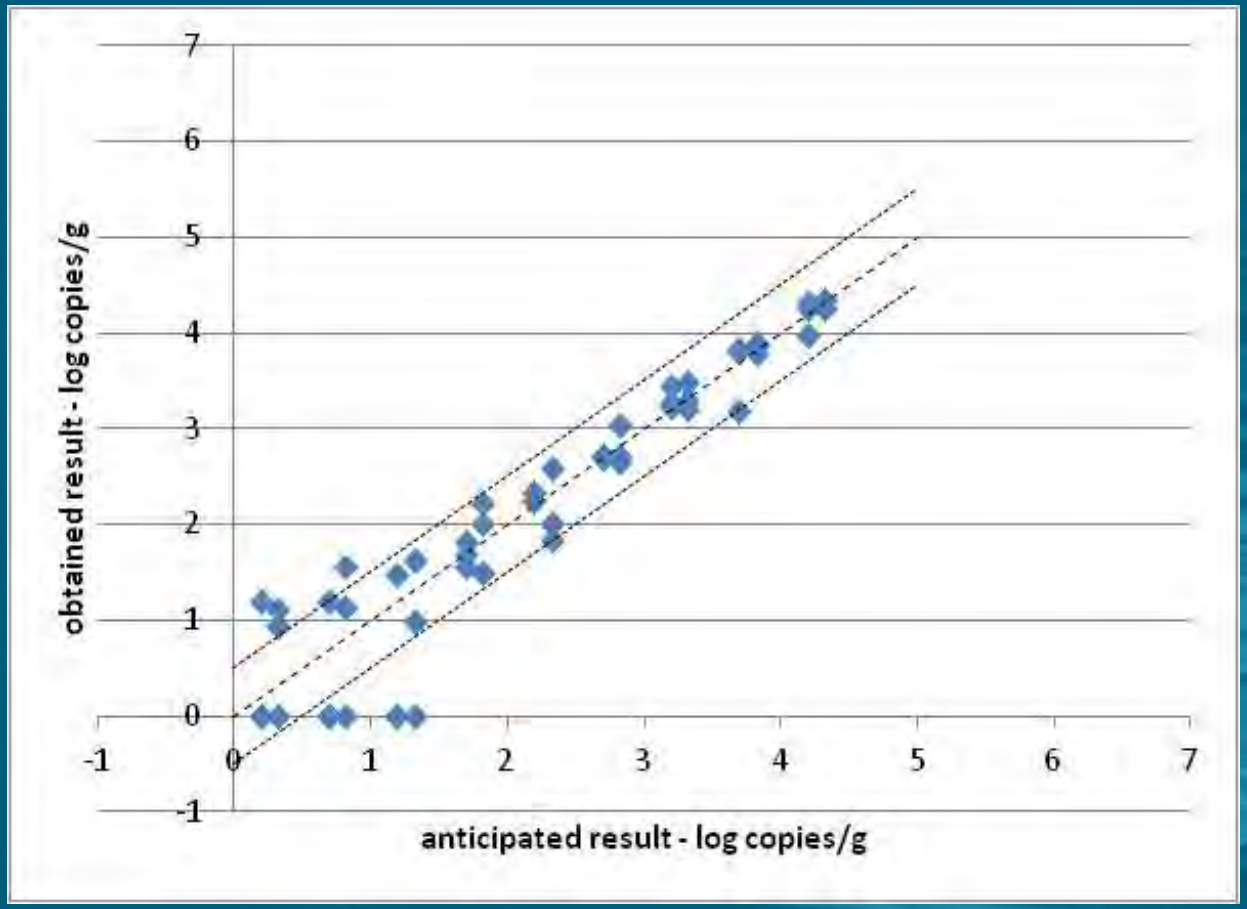
Method characterisation results

- Quantification of norovirus GI in oysters



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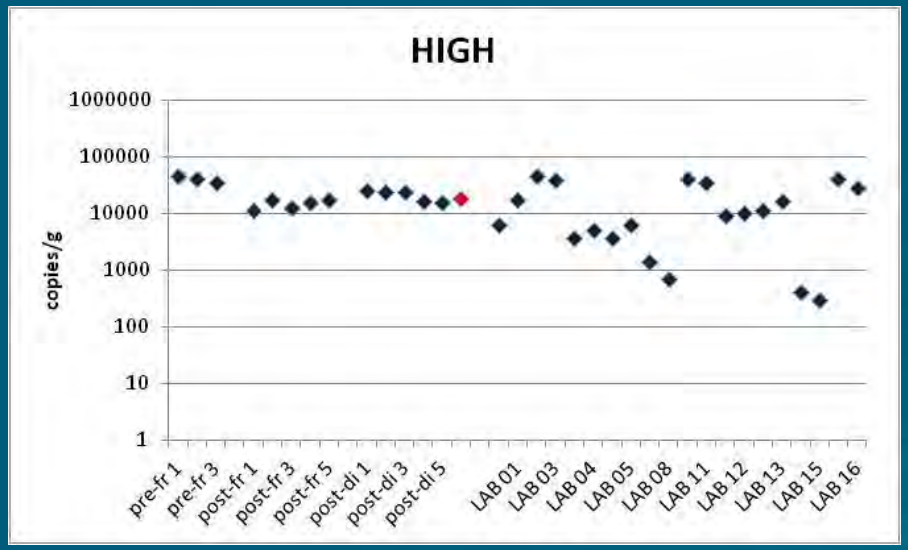
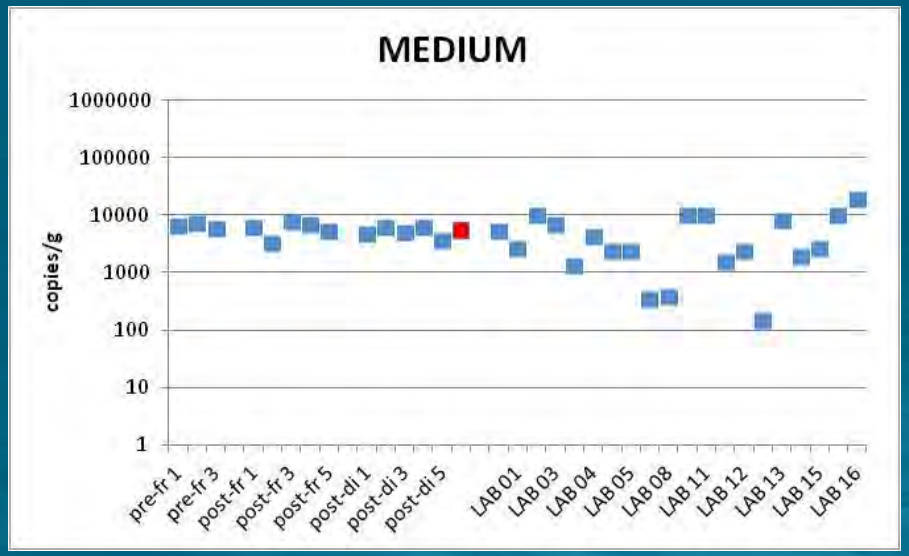
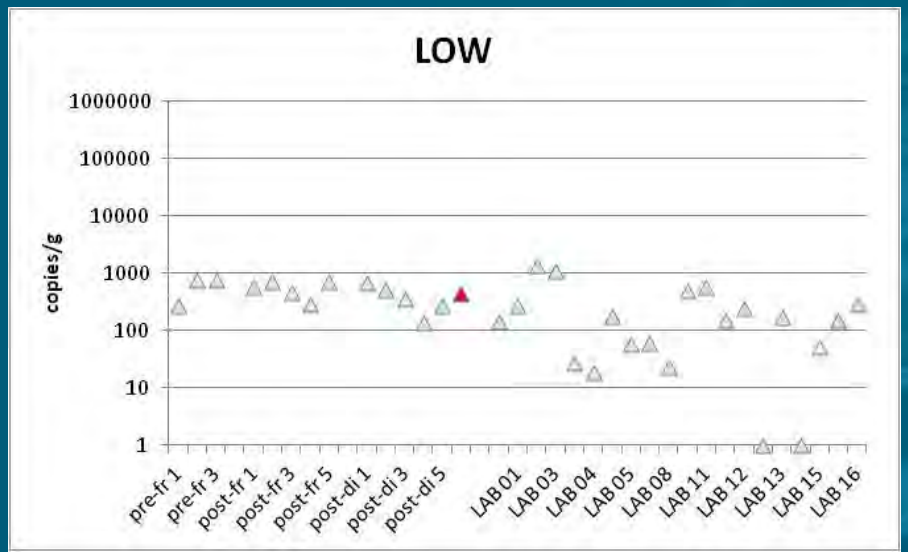
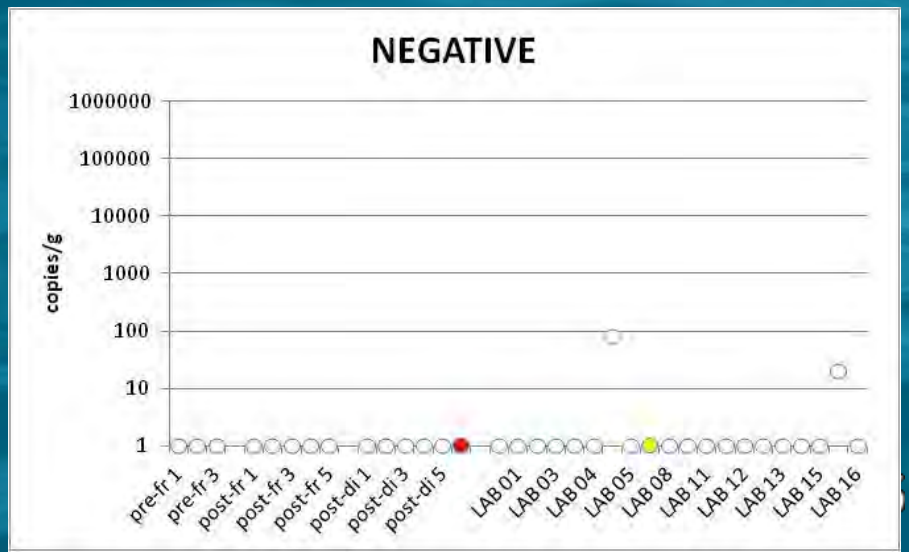
Inter-laboratory trial results

- Quantification of norovirus GI in oysters



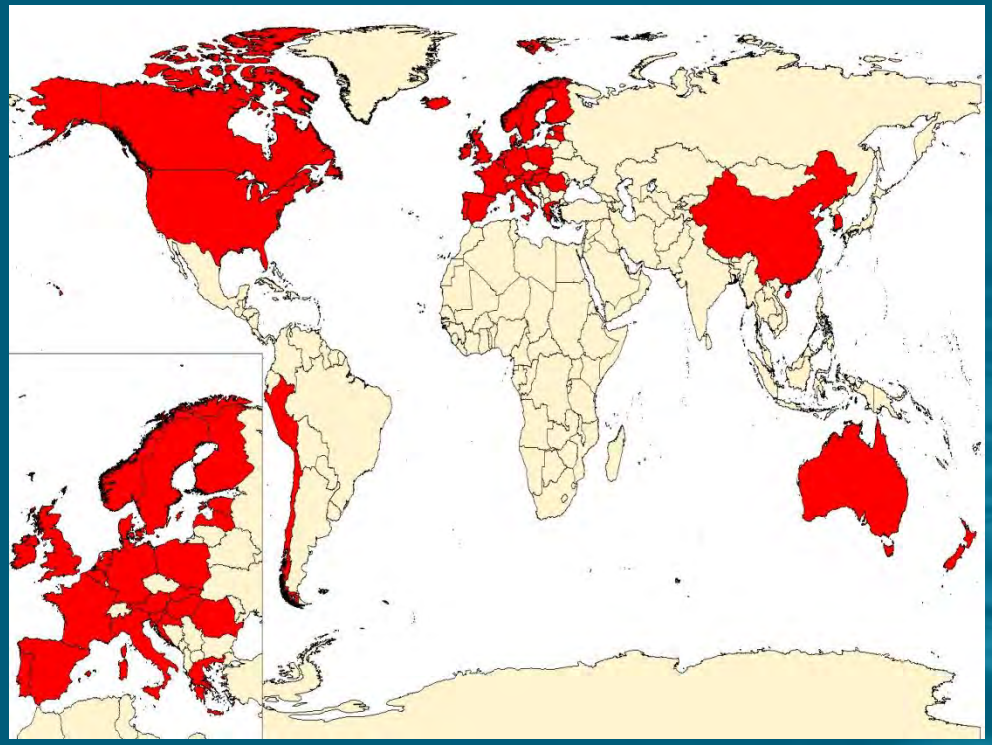






EURL virus proficiency testing

- World wide
- 13 distributions
- 33 countries
- 42 labs participated in 2013



Year	Report No.	Norovirus	Hep A virus	Whole animal	Lenticule
2003	RT 3		✔		
2004	RT 7	✔		✔	
2005	RT 10	✔	✔		
2005	RT 15	✔	✔	✔	
2006	RT 19	✔	✔		✔
2008	RT 25	✔	✔		✔
2009	RT 27	✔	✔		✔
2009	RT 33	✔	✔		✔
2011	RT 39	✔	✔	✔	✔
2011	RT 43	✔	✔	✔	✔
2012	RT 46	✔	✔	✔	✔
2013	RT 50	✔	✔	✔	
2014	RT 53	✔	✔	✔	✔

- Australia
- Austria
- Belgium
- Canada
- Chile
- China
- Croatia
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece
- Hungary
- Iceland
- Ireland
- Italy
- Korea
- Latvia
- New Zealand
- Norway
- Peru
- Poland
- Portugal
- Romania
- Singapore
- Slovakia
- Slovenia
- Spain
- Sweden
- Netherlands
- UK
- USA

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EURL virus proficiency test

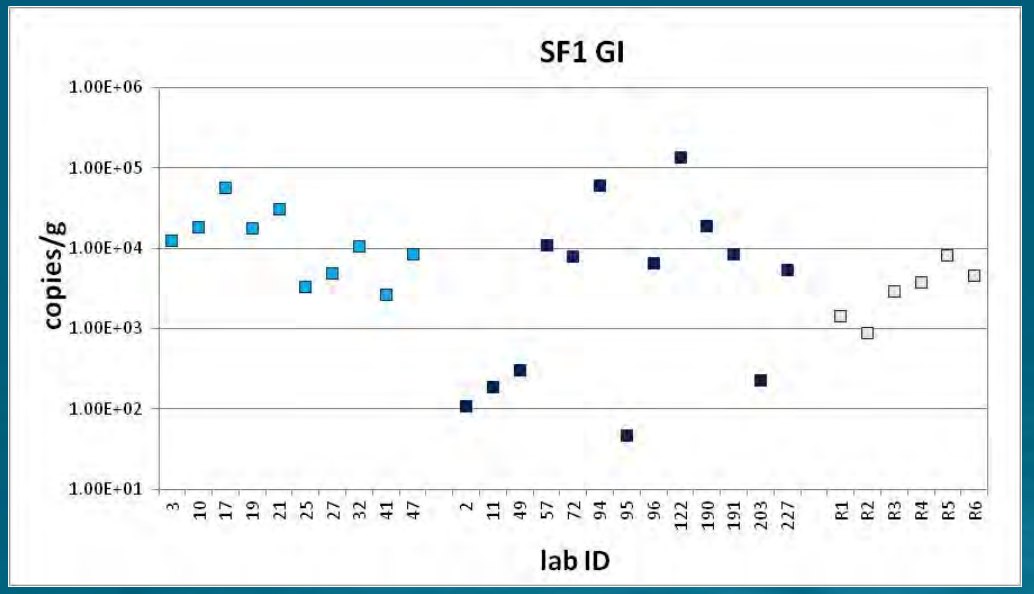
Participation 2013

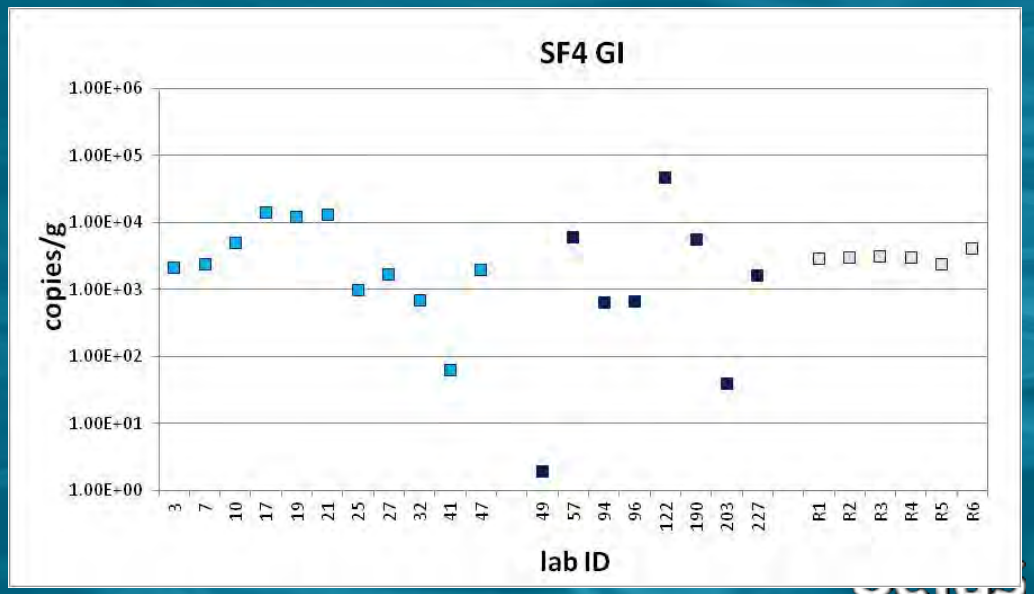
PT 50 - Virus	
Country	
Australia	1
Austria	NRL
Belgium	NRL +1
Canada	2
Chile	1
Denmark	NRL
Finland	1
France	NRL +2
Germany	NRL +1
Ireland	NRL
Italy	NRL
Korea	1
Netherlands	NRL +2
New Zealand	1
Norway	1
Peru	4
Poland	NRL +1
Portugal	NRL
Romania	NRL
Singapore	1
Slovakia	NRL
Slovenia	NRL
Spain	NRL + 5
Sweden	NRL
UK	NRL + 1

Virus (norovirus and HAV)

EU MS	17
MS NRLs	16
EFTA countries	1
Total EFTA country lab	1
total EU+EFTA labs	31
third countries	7
total 3rd country labs	11
	42

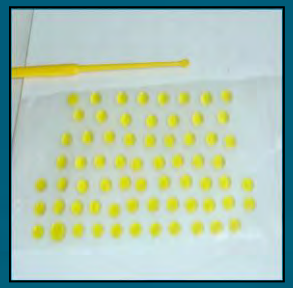
Quantification of norovirus GI in oysters (2013)

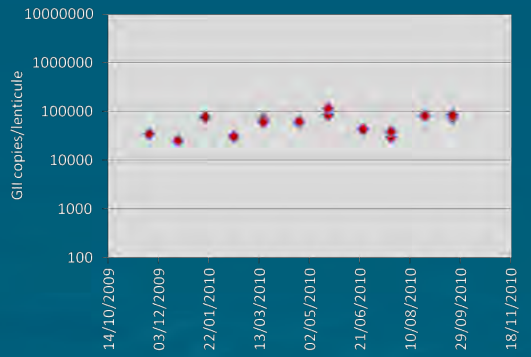


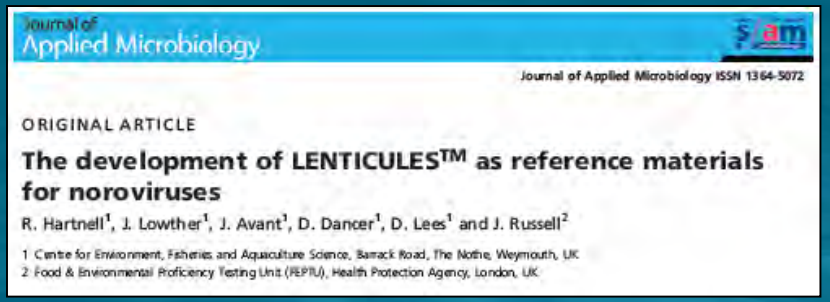


Quality assurance - standard reference materials

- Not previously available, needed for adoption of methodology
- Developed norovirus (GI and GII), HAV lenticules as control materials
- Homogeneity, stability, titre demonstrated to ISO standards
- Lenticules now available commercially in collaboration with Public Health England
- Certificate of analysis (including titre)






Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

The development of LENTICULES™ as reference materials for noroviruses

R. Hartnell[1], J. Lowther[1], J. Avant[1], D. Dancer[1], D. Lees[1] and J. Russell[2]

1 Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, The Nothe, Weymouth, UK
2 Food & Environmental Proficiency Testing Unit (FEPTU), Health Protection Agency, London, UK

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Norovirus in oysters: methods, limits and control options (2012)

efsa
European Food Safety Authority
Committed *since 2002* to ensuring that Europe's food is safe

Control options – post harvest interventions

- Depuration not reliable for viruses (as currently performed)
- Relaying may be effective – but requires >4 weeks
- Cooking is effective – but only when commercially controlled
- High pressure processing (to inactivate norovirus) alters organoleptic properties
- Most effective control measures is to prevent virus contaminated molluscs entering food chain

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EFSA 2012

- Variety of PCR based methods are available (reviewed)
- However, proficiency testing demonstrates methodology and QC is critical for comparability (particularly quantitative)
- Standardisation undertaken by European working group (since 2004)
- Standard ISO/CEN method suitable for use in legislation

Limits: infectivity and dose response

- PCR detects both infectious and non-infectious virus particles
- Growing evidence of a dose response i.e. infectious risk increases with dose (as measured by PCR)
  - In clinical studies (Teunis *et al*., 2008)
  - In restaurant study (Lowther et al., 2010)
  - In outbreak samples (EFSA report, Lowther et al., 2012)
- 'infectious risk associated with low level positive oysters as determined by real-time PCR may be overestimated'
- So ..... although cannot determine safe limit can make risk management decision on a control limit (impact vs public heath gain)
- Since indirect measure of risk sum GI and GII

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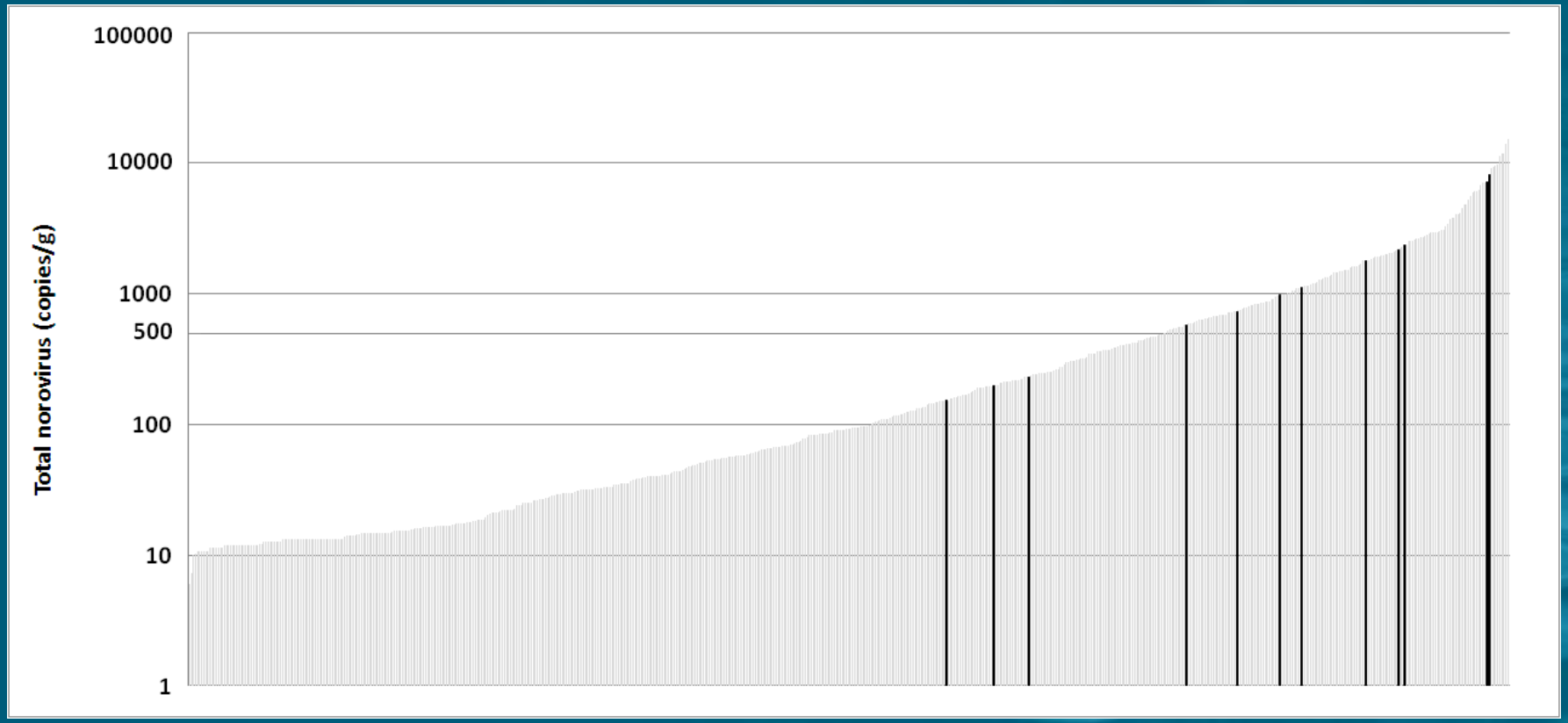
Norovirus levels in outbreak-associated batches of oysters

- All positive samples from 2007-date ranked by norovirus quantity; outbreak samples in black (Lowther et al, J Food Prot. 2012; 75:389)
- Geomean outbreaks (1,048) vs non-outbreaks (121) – statistically significant difference
- No outbreak sample <152 copies per gram



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Impact of limits - Surveillance studies

- Qualitative studies show range of positivity in production area (7-57%) and retail (4-59%) samples
- But different methods
- Few quantitative studies using standardised methods
- EFSA reviewed available production area data from UK, France, Ireland

Production area surveillance data – findings

- All sites were classified and available for commercial harvest
- High level of positivity in all countries (>30%)
- Contamination range observed <100 to 10,000 RNA copies per gram
- Strong winter seasonality
- Absence standard would have a high impact
- Quantitative standard?

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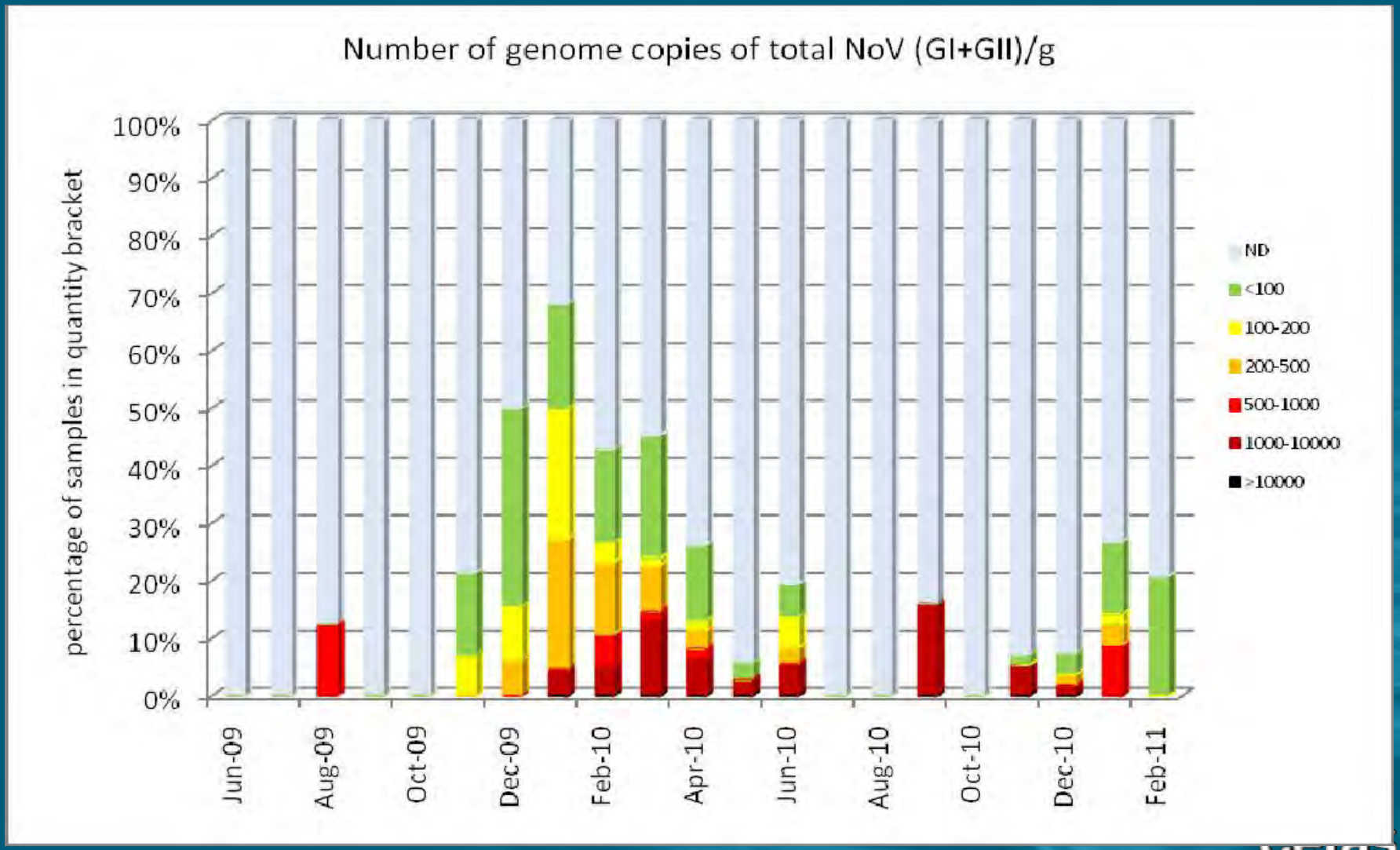
EFSA opinion: Quantitative data vs possible limits France



EFSA: impact of potential limits for samples from commercial production areas

Table 8: Average percentage of samples that would fail during the high risk season (January to March 2010) if a maximum limit of 100, 200, 500, 1000, or 10,000 genome copies/g were set

	100 c/g	200 c/g	500 c/g	1,000 c/g	10,000 c/g
United Kingdom	65.6%	61.1%	46.9%	37.2%	2.7%
Ireland	83.3%	83.3%	72.2%	44.4%	11.1%
France	33.6%	24.4%	10.0%	7.7%	0%

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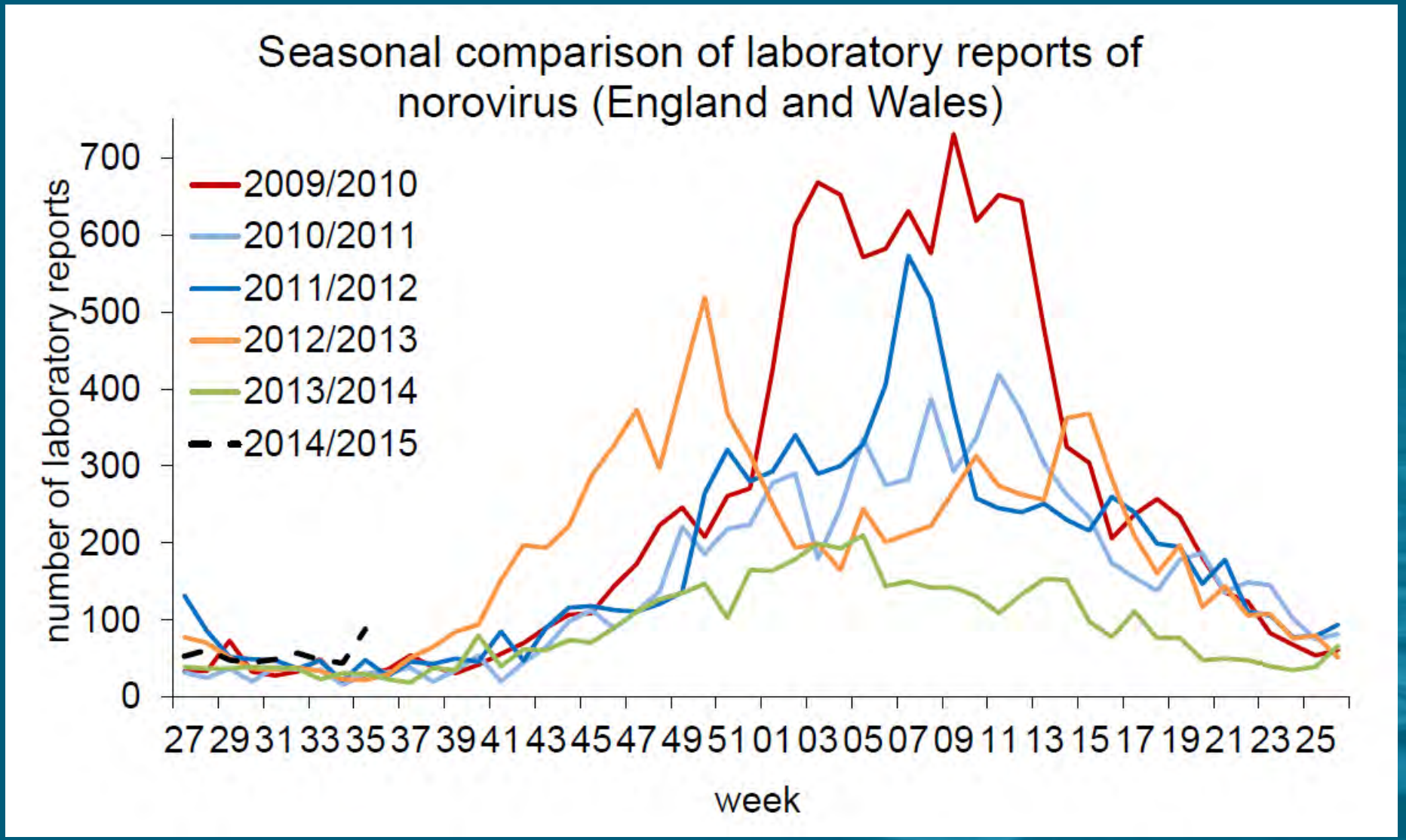
Public Health England – monthly norovirus report



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EFSA conclusions and recommendations

- Virus methods are available and considered suitable for use in legislation
- Dose dependant probability of becoming ill (dose response)
- Relationship between RNA titre and number of infectious particles may not be a constant – indirect measure of risk
- Risk managers should consider establishing virus limits for high risk LBMs (i.e. those consumed raw)
- Post harvest treatments should be validated for effectiveness against viruses

Further information-EU-RL website – www.eurlcefas.org

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Thank you

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