Methods for detection of enteric viruses in food
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

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

- EURL responsible for analytical methods used in Official Controls

Rapid Communications

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

T Westrell, V Dusch, S Ethelberg, J Harris, M Hjertqvist, N Jourdand, da Silva, A Koller, A Lenglet, M Lisby, L Vold

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.
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
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
Digestive gland dissection

- Proteinase K digestion of chopped glands
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
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
Quantitation using standard curve

- Reporting in genome copies per gram of matrix tested
• Set QC criteria for: inhibition and recovery
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
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
Method characterisation results

- Quantification of norovirus GI in oysters
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

<table>
<thead>
<tr>
<th>Year</th>
<th>Report No.</th>
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<th>Hep A virus</th>
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EURL virus proficiency test

Participation 2013

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<td>Canada</td>
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<td>Chile</td>
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<td>Denmark</td>
<td>NNL</td>
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<td>Finland</td>
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<tr>
<td>Germany</td>
<td>NNL +1</td>
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<tr>
<td>Ireland</td>
<td>NNL</td>
</tr>
<tr>
<td>Italy</td>
<td>NNL</td>
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<td>Korea</td>
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<td>Poland</td>
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<td>Portugal</td>
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<td>Singapore</td>
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Quantification of norovirus GI in oysters (2013)

<table>
<thead>
<tr>
<th>Virus (norovirus and HAV)</th>
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<tr>
<td>EU MS</td>
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<tr>
<td>MS NRLs</td>
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<tr>
<td>EFTA countries</td>
</tr>
<tr>
<td>Total EFTA country lab</td>
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<tr>
<td>total EU+EFTA labs</td>
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<tr>
<td>third countries</td>
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<tr>
<td>total 3rd country labs</td>
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</table>
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)
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
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 health gain)
• Since indirect measure of risk sum GI and GII
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
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?
EFSA opinion: Quantitative data vs possible limits
France

Number of genome copies of total NoV (GI+GII)/g

- ND
- <100
- 100-200
- 200-500
- 500-1000
- 1000-10000
- >10000

Percentage of samples in quantity bracket

Jun-09, Aug-09, Oct-09, Dec-09, Feb-10, Apr-10, Jun-10, Aug-10, Oct-10, Dec-10, Feb-11
EFSA: impact of potential limits for samples from commercial production areas

<table>
<thead>
<tr>
<th></th>
<th>100 c/g</th>
<th>200 c/g</th>
<th>500 c/g</th>
<th>1,000 c/g</th>
<th>10,000 c/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>65.6%</td>
<td>61.1%</td>
<td>46.9%</td>
<td>37.2%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Ireland</td>
<td>83.3%</td>
<td>83.3%</td>
<td>72.2%</td>
<td>44.4%</td>
<td>11.1%</td>
</tr>
<tr>
<td>France</td>
<td>33.6%</td>
<td>24.4%</td>
<td>10.0%</td>
<td>7.7%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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.
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
Thank you