Guidance note on assessment of virus testing laboratories for Food Business Operators and Competent Authorities

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1 Purpose and scope

The increasing recognition of viral contamination of bivalve molluscs as a potential health risk to consumers has led to some Food Business Operators in the EU to consider virus testing as a supplementary control measure within their HACCP plans. As a result of this demand an increasing number of laboratories have started to offer virus testing on a commercial basis. It has been recognised that, in the current absence of legislation on virus testing, guidance from the EURL on criteria for selection of virus testing laboratories, designed for industry and other stakeholders (e.g. EU Member State Competent Authorities) would be desirable (see Resolution 20 of the 16th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs). This guidance note therefore considers the current position on laboratory testing for viruses both in the EU and internationally and makes recommendations on best practice regarding laboratory methodology and quality assurance.

2 Background

Contamination of bivalve shellfish with norovirus (causing gastroenteritis) and hepatitis A virus (HAV) is recognised as the major human health risk associated with consumption of faecally contaminated shellfish. High risk factors for shellfish-related norovirus include cold weather (low water temperatures), high prevalence of norovirus gastroenteritis in the community, and high rainfall (leading to sewage system overflows). Risk factors for hepatitis A virus are less well defined.

Risk management for bivalve molluscs aimed at control of faecal pollution risks currently relies heavily on the use of E. coli as an indicator of faecal (sewage) contamination and is enacted under European food regulations (Regulation 854/2004, Anon. 2004a). However, although these regulations probably reduce the burden of infection, particularly for bacterial pathogens, they are not currently viewed as adequately controlling the virus risk. Particular problems are the greater robustness of viruses in the environment, and their different behaviour within bivalve molluscs, compared with bacterial faecal indicators. In the large majority of EU outbreaks shellfish are extracted from officially classified waters, are depurated in approved plants in compliance with requirements, and are processed in approved establishments i.e. they are produced in compliance with the EU legislation. End-product testing will generally also show products to be in compliance with the regulatory E. coli standard. These factors, and the importance of virus as a cause of bivalve shellfish associated human illness, is well recognised by both producers and officials.

These problems have focused attention on the need for development of methods for direct detection in bivalve molluscs of the viruses causing illness (principally norovirus and HAV) and, over the last two decades, considerable progress has been made in this regard, with a two part EN ISO International Standard Method for detection and quantification of viruses in foods (including shellfish) first published in 2013 and updated with validation data in 2017 (ISO 15216). EU legislative text foreshadows the adoption of virus controls when the
methods are available for use (Regulation 2073/2005, Anon., 2005a) and the possible introduction of such controls is a matter of active discussion at international level. However, at the current data, no legislative standard for viruses in shellfish exists.

Nevertheless, despite the absence of a legislative framework or standardised methods, virus testing may be desirable for Food Business Operators, retailers or Competent Authorities for a number of reasons:-

- **Producer HACCP programmes.** Following outbreaks, or other suspicion of virus contamination, virus testing may be a response to a requirement on producers to document the adequacy of measures to control virus contamination.

- **Producer own-checks.** Following suspect or confirmed virus outbreaks, or for due diligence purposes, producers may seek virus testing to document virus status of their products. Producers sourcing from several suppliers may also utilise virus testing to inform their stock purchasing decisions.

- **Mandatory certification requirements for exports.** Some overseas markets (eg Hong Kong and Singapore) have in the past introduced product certification requirements for absence of norovirus.

- **Human illness incidents of suspect or confirmed viral aetiology.** Virus testing of implicated shellfish can assist investigations into the responsible food vehicle and the agent aetiology. Investigation of virus contamination in the attributed production area can contribute to risk assessment.

- **Follow up investigations and checks following outbreaks.** Virus surveillance in implicated production areas can help to ascribe ongoing risk and can help pinpoint sources of virus contamination.

- **Responses to food alerts (eg RASFF).** Official and/or producer responses to food alerts on exports (either outbreaks and/or overseas laboratory testing) may incorporate virus testing.

### 3 International Method standardisation

The Microbiology of the Food Chain working group within the European Committee on Normalisation (CEN) convened a Technical Advisory Group on Viruses in Food in 2004. This group, CEN/TC275/WG6/TAG4, currently consists of approximately 40 international experts in virology from 30 institutions in 15 countries and is led by scientists from the EURL. The group was tasked from its inception with development of an International Standard Method for detection and quantification of viruses (including norovirus and hepatitis A virus) in foods (including bivalve shellfish). In 2013 the two-part standard method developed by the group was first published jointly by CEN and the International Standards Organization (ISO) as


At this stage the method included no validation data and was published as a Technical Specification. An International method validation project involving 18 laboratories from 11
European countries (including the EURL and six other NRLs within the live bivalve mollusc network) was conducted between 2012-2014. Method characteristics generated from this project were incorporated into a revised version of 15216-1, published as a full standard in 2017 (ISO 15216-1:2017, Anon., 2017b). Currently work is ongoing on a revision to 15216-2, with republication as a full standard including method characteristics anticipated in 2019.

It is anticipated that should EU Regulations adopt a virus standard in the future then the ISO 15216 method will be specified as the reference method. Other methods could be employed but would need to be shown to give equivalent performance though comprehensive validation studies conducted in accordance with international rules (ISO 16140-2, Anon., 2016). Given the likely future legal status of the ISO 15216 method (as a reference method), and the high level of European and wider international expertise applied to the method development and evaluation, it is recommended that, where possible, the methodology in ISO 15216 is used by testing laboratories.

The EURL has produced a generic protocol for quantification of viruses in bivalve shellfish (available at https://eurlfac.org/public-documents/methods.aspx) that is based on ISO 15216-1, but which includes many specific methodology and reagent recommendations that are not mandatory according to ISO 15216 (although many are described in the informative annexes of the ISO document).

4 Methodology recommendations

Methods employing variants of the polymerase chain reaction (PCR) for the detection of viruses in shellfish have been published since the mid 90’s (Lees, 2000) and there are now numerous published references from laboratories world-wide. It is important to note that all published methods with demonstrable ability to detect viruses in bivalve shellfish or other foods have utilised PCR. Thus methods based on other possible approaches available for clinical samples (eg ELISA) have not been demonstrated to have adequate sensitivity for foods and should not be used. Any proposals to use such methods would particularly need to demonstrate adequate sensitivity for detection of viruses in environmentally contaminated samples and equivalence to the reference (ISO 15216) method according to the ISO 16140 series of standards on method validation (Anon., 2016) if they were to be applied in an Official Control context.

4.1 General requirements for operation of PCR laboratories and tests

PCR is an extremely sensitive technique and is well known to be susceptible to cross-contamination events within the laboratory and also to matrix interferences causing PCR inhibition. Hence the potential for both false positive and false negative results are well documented. The application of PCR to food testing requires significant investment by laboratories for both staff and equipment to ensure that analysis can be performed to a satisfactory standard. Laboratories should conduct PCR-based testing in a laboratory environment consistent with internationally agreed guidance. This has significant resource implications such as the need to physically separate pre- and post-PCR activities to avoid cross-contamination. Laboratories not conforming to the physical separation requirements are highly likely to experience false positive test results at some point. ISO have considered the laboratory and general testing requirements for analysis of food samples using PCR based methods (ISOs 20836 [Anon., 2005b], 20837 [Anon., 2006a], 20838 [Anon., 2006b], 22118 [Anon., 2011a], 22119 [Anon., 2011b], 22174 [Anon., 2005c] etc.) and these general
requirements should be carefully evaluated and complied with. Compliance with the general ISO guidance is considered as a prerequisite in the performance of ISO 15216.

4.2 Virus target
The principal viruses of concern in the EU and internationally are norovirus and hepatitis A virus. It is recommended that methods for bivalve shellfish specifically target these viruses. Norovirus is currently grouped into seven genogroups of which two, norovirus genogroup I (GI) and norovirus genogroup II (GII) cause the vast majority of human infections. Assays should target both genogroups as they are both commonly responsible for human infection and are both common contaminants of bivalve shellfish in EU waters.

4.3 Extraction procedure
Viruses are present in bivalve shellfish at low to very low levels compared with human clinical samples. However, unlike most enteric bacteria causing food-poisoning, enteric viruses can initiate an infection at very low levels. For example the infectious dose for norovirus is generally accepted to be around 10 infectious particles. Thus the low levels of virus generally found in bivalve shellfish have been demonstrated to pose a health risk (Bosch et al., 1994; Sánchez et al., 2002, Le Guyader et al., 2003, 2006a). An additional complication is that PCR is very susceptible to food matrix inhibition causing false negative reactions. This has been shown in the past to be a particular problem with bivalve shellfish. Norovirus and environmental strains of hepatitis A virus cannot be routinely grown in cell culture and thus biological amplification, a potential solution to these problems, cannot be employed. Consequently the matrix extraction procedure is critical and laboratories should be able to demonstrate that their methods are capable of recovery of low levels of contaminating virus at a purity consistent with PCR requirements (i.e. not inhibitory).

Most methods in use internationally now focus on the dissected bivalve digestive tissues as the starting material for virus extraction. This organ has been shown to be the focus of contamination within the bivalve (Metcalf et al., 1980, Romalde et al., 1994) and research suggests that this may be due to specific receptors within the digestive tissues (Le Guyader et al., 2006b). Digestive tissues comprise approximately 10% of the body mass of the bivalve but contain the large majority of the contaminating virus. Thus, targeting the digestive tissues avoids the need to process tissues containing little virus but many PCR inhibitors. This reduces processing time and aids both sensitivity and the quality of the extract. It is recommended that testing laboratories utilise digestive tissues extractions and this is the approach taken in ISO 15216, where a test portion of 2g is stipulated.

The specific method used within ISO 15216 utilizes a solution of the enzyme proteinase K to break down the digestive tissues and release virus particles.

4.4 Nucleic acid purification
Following initial extraction of virus from bivalve tissues all methods require further stages of purification and concentration of virus nucleic acid prior to PCR. This stage is vital for both removal of PCR inhibitors and concentration of virus template to achieve sufficient sensitivity. Many approaches to purification and concentration have been detailed in the literature. For compatibility across a wide range of food-stuffs ISO 15216 used an approach based on the Boom method (Boom et al., 1990). This method utilises guanidine thiocyanate (GITC) to
denature viral coat proteins in combination with silica particles to bind released nucleic acid, which is then purified through successive washing stages before final elution in a small volume. The Boom method principles are also employed by a number of commercial kits for nucleic acid extraction and clean-up. Quality assured commercial reagents can improve intra-laboratory comparability through removing a source of variability. In the generic protocol and the informative annexes of ISO 15216, a method using magnetic silica beads is detailed.

4.5 **Real-time reverse transcription polymerase chain reaction (qRT-PCR)**

The polymerase chain reaction (PCR) is a ubiquitous method within molecular biology to amplify and detect specific DNA sequences using the enzyme DNA polymerase, and short sequence-specific DNA molecules called primers. PCR requires a DNA target or template, however it can be modified to detect RNA sequences, such as the RNA genomes of norovirus and hepatitis A virus, by adding a preliminary stage called reverse transcription, where the enzyme reverse transcriptase makes a DNA copy of an RNA molecule. The two-stage combined process of reverse transcription and PCR is called reverse transcription PCR (RT-PCR).

The earliest publications describing the detection of viruses in shellfish using RT-PCR utilised conventional gel based RT-PCR giving qualitative results (Green et al., 1998; Le Guyader et al., 2000; Formiga-Cruz, et al., 2002; Atmar, et al., 1995). However, subsequently real-time or quantitative RT-PCR (qRT-PCR) was developed as an extension of conventional RT-PCR with significant advantages for application to food monitoring. In qRT-PCR the amplification of the target can be monitored in real-time by monitoring the fluorescence released by a reporter molecule. There are a number of different variants of qRT-PCR; in TaqMan qRT-PCR a probe (another short sequence-specific DNA molecule in addition to the primers) containing a fluorescent dye is broken down by the enzyme DNA polymerase as it amplifies the target sequence, and the dye is released and detected by the qRT-PCR machine.

The advantages of qRT-PCR compared with conventional PCR are that it is much less liable to cause cross-contamination of subsequent samples since tubes do not have to be opened after amplification; it is significantly more efficient logistically; a confirmation step is built into the procedure through the use of labelled probes thus avoiding the need for confirmation of positives through sequencing; it is quantitative; it is standardisable. Because of these significant advantages this type of methodology has been standard for detection and quantification of viruses in foods for more than a decade, and the use of TaqMan qRT-PCR is specifically required by ISO 15216.

4.6 **Primers and probes**

Real-time RT-PCR primers and probes for virus detection in shellfish need to be broadly reactive to ensure that the assay is capable of detecting the diversity of strains seen in the environment. This is a particular problem for norovirus where strain diversity is extremely high. Inappropriate choice of PCR primers or probes (for real-time assays) will negatively impact the performance of the assay. Design and evaluation of virus primers and probes requires specialist knowledge and access to a wide diversity of characterised clinical samples. It is thus not generally possible for food laboratories, in isolation, to develop new PCR primers or probes. Given the technical difficulties in this area it is recommended that commercial laboratories follow the guidance in ISO 15216. According to this document real-
time TaqMan primers and probes must be published in a peer-reviewed journal, must be verified for use against a broad range of strains of the target virus and must target specific regions of the virus genome. For hepatitis A virus primers must target the highly conserved 5’ non-coding region; the primer/probe set as published by Costafreda et al., (2006) is included in the informative annexes of ISO 15216 and the generic protocol. Hepatitis A virus is a well conserved virus in comparison with norovirus and this recommended primer/probe combination is unlikely to require modification through the emergence of, for example, new hepatitis A virus variants. For norovirus the high level of divergence between norovirus GI and GII strains dictates the need for separate assays for these genogroups. A breakthrough occurred with the publication by Kageyama et al., (2003) showing that the ORF1-ORF2 junction region of the norovirus genome (both GI and GII) was the most highly conserved within genogroups and suitable for real-time TaqMan primer design. Most subsequent publications for bivalve applications (Jothikumar et al., 2005; Loisy et al., 2005) have targeted this region with relatively minor modifications of the original proposals, and this is the target area stipulated in ISO 15216. Recommended primer and probe sets for both GI and GII norovirus are included in the informative annexes of ISO 15216 and the generic protocol, including a choice of probes for GI. At the present time these primers and probes remain suitable for detection of a very wide spectrum of strains including all the most commonly occurring, however it is possible that as new variants emerge that different primer/probe selections may be more appropriate.

4.7 Quantification

Real-time RT-PCR has a major advantage over conventional RT-PCR in that it can be used to quantify to concentration of virus target in a sample. In qRT-PCR, fluorescence is measured throughout the reaction, and the qRT-PCR cycle at which the fluorescence reaches a threshold (the quantification cycle or Cq value, alternatively called the Ct value) is determined for each reaction. The Cq value depends on the amount of virus present in the shellfish sample; more contaminated samples will have lower Cq values. By comparison with known standards (see below) Cq values are converted into sample concentrations in virus genome copies/g of digestive tissue tested. In negative samples, no amplification occurs, the fluorescence level never reaches the threshold, and no Cq value is produced.

4.8 Controls

Given the need for a highly sensitive PCR to detect the low levels of norovirus and hepatitis A virus found in environmentally contaminated shellfish samples, and the susceptibility of such a sensitive PCR to cross-contamination (false positives) and also matrix interference (false negatives), it is vitally important to incorporate alongside each test sample batch an appropriate suite of controls. ISO 15216-1 incorporates the following controls:-

- negative RT-PCR control
- negative RNA extraction control
- positive PCR and quantification control
- positive RT-PCR control
- RT-PCR inhibition control
• process control for extraction efficiency

The negative RT-PCR control, negative RNA extraction control, positive PCR and quantification control, positive RT-PCR control and RT-PCR inhibition control are tested for each target assay performed i.e. separately for norovirus GI, norovirus GII, and hepatitis A virus, while the process control uses a separate assay for a non-target virus.

The negative RT-PCR control consists of aliquots of molecular biology grade water tested alongside the samples on the real-time RT-PCR run. The negative extraction control is an aliquot of molecular biology grade water subjected to RNA Extraction and real-time RT-PCR in parallel with the samples. The positive PCR and quantification control is a dilution series of a DNA molecule including the PCR target region subjected to real-time RT-PCR alongside the samples. This simultaneously allows confirmation of successful amplification of target in the PCR element of the RT-PCR, and enables quantification of the samples as described above. The positive RT-PCR control is an RNA molecule including the PCR target region subjected to real-time RT-PCR alongside the samples, which allows confirmation of successful reverse transcription of RNA target into DNA. The same RNA molecule when added to the sample extracts, acts as the RT-PCR inhibition control enabling assessment of disruption of the RT-PCR process by inhibitory substances in the extracts on a sample-by-sample basis. The process control allows the assessment of the recovery of virus (extraction efficiency) during the whole virus and RNA extraction using a related RNA virus spiked into the test sample at the beginning of the extraction process then tested in parallel with the target viruses. Comparison of spiking material concentration and final extract concentration determines the extraction efficiency of the whole process. ISO 15216 includes criteria for selection of process control viruses and the informative annexes recommend the use of Mengo virus strain MC0 as described by Costafreda et al (2006). Mengo virus is a suitable analogue for both hepatitis A virus and norovirus thus avoiding the need for two separate process controls. The use of a process control gives information both about the suitability of the extraction in general (reagent batches, operator technique, etc) and also specific information about the success in extraction of the test sample.

The laboratory should develop quality assurance acceptance criteria for each of the above controls which will determine the acceptability of each test and batch run.

5 Calculation of results and reporting
5.1 Quantitative methods

Conversion of raw data in terms of Cq values into meaningful sample concentrations is a complex multi-stage procedure. For this reason results have historically been reported by some laboratories in terms of Cq values only. However there are a number of technical reasons why this type of reporting is limited in value (summarised in a UK NRL guidance document (https://www.cefas.co.uk/media/1559/norovirus-ct-values-vs-copies-per-gram-cefas-jan-2012.pdf). Principally, comparison of results between laboratories is extremely difficult, and even within laboratories run-to-run variations and arbitrary choices around selection of analysis parameters may render comparisons difficult across a time period. For these reasons, ISO 15216-1 stipulates reporting of results in terms of concentration in the starting material, with reporting units of detectable virus genome copies per gram digestive tissues (copies/g). ISO 15216-1 and the generic protocol both detail the necessary calculations for generating these figures from the raw data, through the use of the standard
curve. In addition, the EURL has produced a spreadsheet for use with the method in ISO 15216-1, available at https://eurlocefas.org/public-documents/methods.aspx.

To report results consistently with ISO 15216-1, it is necessary for the reporting laboratory to determine both the limit of quantification (for reporting low level positive results) and the limit of detection (for reporting not detected results) of the method as applied in their laboratory, in order to properly contextualise the results. The ISO document does not specify the method to be used for the generation of these method characteristics, however the EURL has provided guidance on one possible method to use, available at https://eurlocefas.org/public-documents/methods.aspx. The extraction efficiency (as described in 4.8) should also be included on the test report.

5.2 Qualitative methods

To report the results of qualitative analyses consistently with the ISO, results should be reported as either “virus detected in x g” or virus not detected in x g” where x is the weight of the test portion (2g if following ISO 15216-2). The extraction efficiency (as described in 4.8) should also be included on the test report.

6 Result interpretation (infectivity)

A major result interpretation issue is that it is not clear whether presence of virus genome, as determined by PCR, correlates with a human health risk. It is possible that, in some cases, PCR may be detecting inactivated or non-infectious virus. If virus presence were an infrequent occurrence in bivalve shellfish this would not present a significant practical problem. Unfortunately, although hepatitis A virus contamination is relatively infrequent, for norovirus this does not appear to be the case - particularly for class B areas. Current data both from EU Member States shows that (particularly during winter months) norovirus presence in shellfish is a fairly common occurrence, with >35% prevalence recorded in several surveys (e.g. Flannery et al., 2009, Lowther et al., 2012b, Polo et al., 2015, Suffredini et al., 2014). The prevalence of norovirus positives from such studies exceeds our expectation of likely disease burden based on human health incidents reported. The possibilities are either that disease reporting dramatically underestimates the actual disease burden or that PCR testing over estimates the real risk – or a combination of both factors. There is some evidence that for norovirus in shellfish, human health risk is correlated with levels as determined by PCR (Lowther et al., 2010, Lowther et al., 2012a), and that quantitative PCR testing therefore provides an adequate indication of risk. However, expansion of the methodology to include direct or indirect measurements of infectivity would be beneficial, and much effort has been directed at this (reviewed in Knight et al., 2012). At the present time, no proposed method has shown suitability for routine use, however, and the best method for assessment of viruses in shellfish remains qRT-PCR.

7 Quality assurance

Given the technical complexity in this area, and the potential for both false positive and false negative results, it is vital that laboratories endeavour to comply with internationally accepted quality assurance criteria.
7.1 **Accreditation**

Accreditation to the ISO 17025 standard (Anon., 2017c), or working towards accreditation to this standard, is required of laboratories performing official control testing under EU Regulation 882/2004 (Anon., 2004b) and its forthcoming replacement Regulation 2017/625 (Anon., 2017a). It is highly recommended that laboratories carrying out testing for industry stakeholders carry specific accreditation to ISO 17025 for this test.

7.2 **Reference materials**

The absence of external certified or standardised reference materials for viruses poses a problem for laboratories in ensuring traceability of assay measurements to known reference values. However, in collaboration with Public Health England (PHE), the EURL has developed stable extraction positive control materials (LENTICULE discs) with known reference values for norovirus and hepatitis A virus. Further details are available at [https://www.phe-culturecollections.org.uk/news/ncpv-news/reference-materials-for-norovirus-and-hepatitis-a-virus.aspx](https://www.phe-culturecollections.org.uk/news/ncpv-news/reference-materials-for-norovirus-and-hepatitis-a-virus.aspx). In addition, synthetic norovirus RNA sequences can be purchased e.g. from ATCC (see [https://www.lgcstandards-atcc.org/Products/Nucleic_Acid_Proteins_and_Cell_Extracts/Synthetic_Nucleic_Acids/](https://www.lgcstandards-atcc.org/Products/Nucleic_Acid_Proteins_and_Cell_Extracts/Synthetic_Nucleic_Acids/)) or Microbiologics (see [http://www.microbiologics.com/item-type/Product/product-format/Helix-Elite-Genomic-Extract,Helix-Elite-Inactivated-Standard,Helix-Elite-Synthetic-Standard/](http://www.microbiologics.com/item-type/Product/product-format/Helix-Elite-Genomic-Extract,Helix-Elite-Inactivated-Standard,Helix-Elite-Synthetic-Standard/)). It is recommended that testing laboratories incorporate testing of those materials that are available into the quality systems.

7.3 **Proficiency testing (PT)**

Proficiency testing provides an external assessment of laboratory and test performance through blind analysis of samples distributed by a PT scheme organiser. Results for all participants are reported together to enable laboratories to compare their results both with other laboratories and with the reference results provided by the scheme organiser. Given the technical difficulties of testing bivalve shellfish for viruses, and the absence of other means to ensure accuracy of test results, it is considered vital that laboratories endeavour to participate in external quality assurance or proficiency testing (PT). Evidence of satisfactory performance should be given to the laboratory customer. There are currently very few PT schemes available for virus testing in shellfish. The EURL runs an international PT scheme using matrix samples for National Reference Laboratories that is also available to other laboratories on a cost-recovery basis (details at [https://eurlcefas.org/public-documents/proficiency-testing/eurl-pt-programme.aspx](https://eurlcefas.org/public-documents/proficiency-testing/eurl-pt-programme.aspx)). In addition the EURL and PHE collaboratively organise a scheme for virus testing using LENTICULE discs. More details are available at [https://www.gov.uk/government/collections/external-quality-assessment-eqa-and-proficiency-testing-pt-for-food-water-and-environmental-microbiology](https://www.gov.uk/government/collections/external-quality-assessment-eqa-and-proficiency-testing-pt-for-food-water-and-environmental-microbiology).

8 **Best practice recommendations**

Considering the above factors the EURL offers the following recommendations on best practice for laboratory methodology, and quality assurance, in relation to testing bivalve molluscs for viruses:
8.1 **Method.** The only methods currently demonstrated to work at the required sensitivity are based on PCR. Hence methods based on other possible approaches, eg ELISA, are not appropriate. The method detailed in ISO 15216 is considered the most appropriate.

8.2 **Laboratory environment.** PCR is very susceptible to cross-contamination events within the laboratory and to matrix interferences. Hence the potential for both false negative and false positive results is well documented. Laboratories should conduct PCR-based testing in a laboratory environment consistent with internationally agreed guidance such as that published by ISO (see references). This has significant resource implications such as the need to physically separate pre- and post-PCR activities. Laboratories not conforming with the physical separation requirements are highly likely to experience false positive test results.

8.3 **Controls.** Given the sensitivity of PCR and its susceptibility to matrix interferences it is critical to incorporate within each test batch an appropriate suite of controls. The general requirements for PCR controls are given in ISO guidance (see reference list) and the specific controls most appropriate for testing shellfish for viruses are detailed in ISO 15216, including:
- negative RT-PCR control
- negative RNA extraction control
- positive PCR and quantification control
- positive RT-PCR control
- RT-PCR inhibition control
- process control for extraction efficiency

8.4 **Results reporting.** Laboratories should report results in a manner consistent with ISO 15216, e.g. for a quantitative method (following ISO 15216 part 1) high level positive results should be expressed in terms of detectable virus genome copies per gram of digestive tissues (copies/g) while low level positive and not detected results should be reported with reference to the limits of quantification and detection of the method respectively. For a qualitative method (following ISO 15216 part 2) results should be reported as virus genome detected or not detected.

Reporting of results in terms of Cq values only is of limited value, and should not be used to compare results obtained from different laboratories.

8.5 **Test performance evidence.** Detection of low levels of virus template by PCR in a food matrix is known to be very demanding and laboratories should be able to demonstrate to their customers satisfactory evidence of test performance. Laboratories should be able to provide single laboratory test performance data on method linearity, limit of detection, and limit of quantitation (if quantitative methods are used). Such studies should be performed on shellfish matrix material contaminated with virus by a route representative of that occurring in the field (e.g. bioaccumulation). In addition the laboratory should be able to demonstrate applicability of its methods through documenting the presence of norovirus in field samples.
from known polluted areas during winter months (many studies have shown this to be a common occurrence).

8.6 Proficiency testing. The laboratory should endeavour to participate in external quality assurance or proficiency testing (PT) for virus testing in order to compare its performance with that of other laboratories. Evidence of satisfactory performance should be given to the laboratory customer.

9 References


Anon. (2017a). Regulation (EU) 2017/625 Of The European Parliament And Of The Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food


