Food and Agriculture Organization of the United Nations

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Centre for Environment Fisheries & Aquaculture Science

World Class Science for the Marine and Freshwater Environment

Norovirus testing in shellfish – guidance on best practise

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Filter feeding bivalve shellfish species, such as oysters and mussels, can cause human illness when contaminated with certain pathogens. The increasing recognition of viral contamination of bivalves as a potential health risk to consumers has led some Food Business Operators to consider virus testing. It has been recognised that, in the current absence of legislation on virus testing, guidance on criteria for selection of virus testing laboratories, designed for industry and other stakeholders, would be valuable.

This FAO Reference Centre for Bivalve Mollusc Sanitation guidance note provides current information on laboratory testing for viruses and makes recommendations on best practice regarding laboratory methodology and quality of test results.

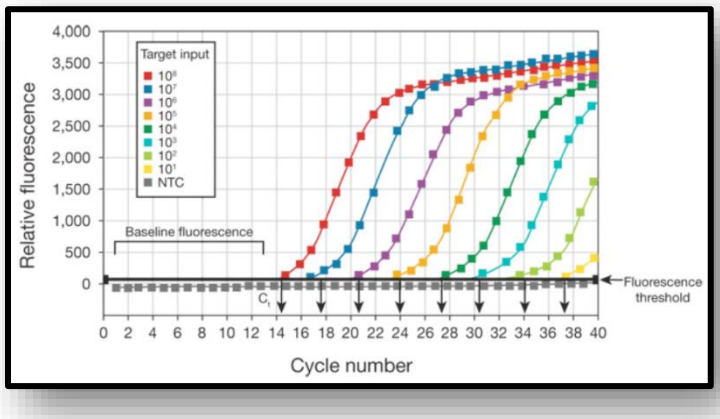
Background:

Contamination of bivalve shellfish with viruses such as norovirus (NoV) and hepatitis A virus (HAV) is recognised as the major human health risk associated with consumption of faecally contaminated shellfish. Risk management for bivalve molluscs aimed at control of faecal pollution risks currently relies heavily on the use of *E. coli* or other bacteria as an indicator of faecal (sewage) contamination and is required under international food safety regulations. However, although these regulations reduce the burden of infection - particularly for bacterial pathogens - they are not currently viewed as adequately controlling the virus risk. In the large majority of outbreaks from shellfish in Europe and elsewhere, the food items originate 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 relevant legislation.

There is therefore a growing commercial need for methods to detect and quantify viruses in bivalve shellfish. Recent developments in testing methods and how they should be used are briefly outlined here. This guidance document provides a check-list for food business operators, retail establishments and regulators to consider when choosing testing methods for NoV and HAV in shellfish.

Available methods

Viruses such as norovirus and hepatitis A can be detected and quantified using appropriate standardized methods, however some key considerations need to be taken into account. A variety of methods employing variants of the polymerase chain reaction (PCR) for the detection of viruses in shellfish have been published since the mid 1990's 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. Methods based on other possible approaches available for clinical samples (e.g. 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.



Key considerations

The FAO Reference Centre makes the following recommendations for best practice for laboratory methodology, and quality assurance, in relation to testing bivalves for viruses:

- ✓ **Method**. The only methods currently demonstrated to work at the required sensitivity are based on PCR. Hence methods based on other possible approaches, e.g. ELISA, are not appropriate. The method detailed in ISO 15216 is considered the most appropriate, and the method for quantification (ISO 15216-1:2017) preferable to a presence/absence method. It is preferable that the laboratory analysis is accredited to ISO 17025.

- ✓ **Laboratory environment**. PCR is very susceptible to cross-contamination events within the laboratory and to inhibition from substances in the tested sample. 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. 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 at some stage.

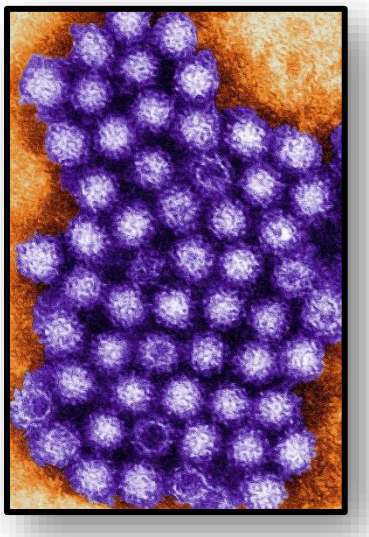
- ✓ **Controls**. Given the sensitivity of PCR and its susceptibility to matrix interferences it is critical to incorporate within each test batch an appropriate suite of controls. The general requirements for PCR controls are given in ISO guidance and the specific

controls most appropriate for testing shellfish for viruses are detailed in ISO 15216, including:-

1. Negative RT-PCR control
2. Negative RNA extraction control
3. Positive PCR and quantification control
4. Positive RT-PCR control
5. RT-PCR inhibition control
6. Process control for extraction efficiency

✓ **Results reporting**. Laboratories should report results in a manner consistent with ISO 15216, e.g. in terms of detectable virus genome copies per gram of digestive tissues (copies/g). Low level positive and not detected results should be reported with reference to the limits of quantification and detection of the method respectively. Reporting of results in terms of cycle threshold (e.g. Ct) values only is of limited value and should not be used to compare results obtained from different laboratories.

✓ **Test performance evidence**. Detection of low levels of virus 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 quantification (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). Ideally, the laboratory should be accredited by an appropriate national accreditation body for the method used (e.g. in the UK this is UKAS, the United Kingdom Accreditation Service) against ISO 17025 as this is an additional and supplementary indicator of laboratory performance.



✓ **Proficiency testing**. The laboratory should 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 if requested.