Current and Emerging Technologies for the Detection of Norovirus from Shellfish

Pradip Gyawali 1,*, Sanjaya KC 2, David J. Beale 3 and Joanne Hewitt 1

1 Institute of Environmental Science and Research Ltd. (ESR), Porirua 5240, New Zealand; joanne.hewitt@esr.cri.nz
2 Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia; sanjaya.kc@imb.uq.edu.au
3 Commonwealth Scientific and Industrial Research Organization, Ecoscience Precinct, Dutton Park, QLD 4102, Australia; david.beale@csiro.au
* Correspondence: pradip.gyawali@esr.cri.nz; Tel.: +64-4914-0700

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Abstract: Reports of norovirus infections associated with the consumption of contaminated bivalve molluscan shellfish negatively impact both consumers and commercial shellfish operators. Current virus recovery and PCR detection methods can be expensive and time consuming. Due to the lack of rapid, user-friendly and onsite/infield methods, it has been difficult to establish an effective virus monitoring regime that is able to identify contamination points across the production line (i.e., farm-to-plate) to ensure shellfish quality. The focus of this review is to evaluate current norovirus detection methods and discuss emerging approaches. Recent advances in omics-based detection approaches have the potential to identify novel biomarkers that can be incorporated into rapid detection kits for onsite use. Furthermore, some omics techniques have the potential to simultaneously detect multiple enteric viruses that cause human disease. Other emerging technologies discussed include microfluidic, aptamer and biosensor-based detection methods developed to detect norovirus with high sensitivity from a simple matrix. Many of these approaches have the potential to be developed as user-friendly onsite detection kits with minimal costs. However, more collaborative efforts on research and development will be required to commercialize such products. Once developed, these emerging technologies could provide a way forward that minimizes public health risks associated with shellfish consumption.

Keywords: norovirus in shellfish; detection methods; emerging technologies; sensing norovirus

1. Introduction

Human fecal contamination from sewage discharge, septic tank leaks/overflows, recreational activities and storm water runoff are major sources of norovirus contamination in coastal waters (i.e., shellfish growing areas) [1]. While environmental (temperature, salinity), physical (dilution, turbidity, transportation) and biological (inactivation) factors can reduce norovirus concentrations in shellfish-growing waters, efficient bioaccumulation in shellfish can result in levels of contamination that pose a risk to consumers as norovirus has a low (~18 viral particles) infectious dose [2].

Norovirus in shellfish can be difficult to remove or inactivate using simple post-harvest treatments such as depuration and relaying [3–5]. As consumers often prefer to eat shellfish either raw or partially cooked, heat and expensive post-harvest treatments such as high-pressure processing are not always viable options for treatment. Therefore, regular microbiological assessment of shellfish growing waters or shellfish is conducted according to regional shellfish quality guidelines. Shellfish harvesting restrictions based on microbiological quality of shellfish growing waters or the shellfish themselves are
Norovirus is a major cause of viral gastroenteritis frequently associated with outbreaks in communal settings such as rest homes, hospitals and restaurants [6,12–14]. Each year norovirus is responsible for approximately 125 million foodborne cases worldwide [15,16], a proportion of which are associated with the consumption of norovirus contaminated shellfish. Accordingly, there is a need for rapid, cheap, reliable and user-friendly detection methods for the onsite/infield detection of norovirus in shellfish to identify the contamination points across the production line (i.e., farm to plate) to reduce public health risk and to minimize shellfish farm harvesting closures. This review evaluates the advantages and disadvantages of currently used methods for the detection of norovirus from shellfish. Furthermore, emerging technologies with the potential to detect norovirus from shellfish are assessed.

Due to the heterogeneous distribution of norovirus in shellfish and the requirement to process a minimum of 2 g of digestive tissue, at least 6–25 shellfish (depending on shellfish size) are used to recover norovirus from shellfish [17–20]. For the recovery of norovirus from shellfish tissue, different methods such as virus elution and concentration, and proteinase K digestion have been developed and evaluated [20–32]. Direct nucleic acid extraction from the digestive tissue of shellfish has also been used [33,34].

For the virus elution and concentration methods, recovery of norovirus (or viral surrogates) usually involves the release of viruses from the digestive tissue of shellfish using buffers (such as phosphate buffered saline, Tris-HCl buffer, alkaline glycine buffer) [20,21,24,30]. The eluted viruses can then be concentrated using polyethylene glycol (PEG) precipitation, ultrafiltration, ultracentrifugation, immuno-concentration or cationic separation (Figure 1). In some cases, the concentrated material is subjected to further clarification such as chloroform extraction, or additional ultracentrifugation.

![Figure 1](image-url)

**Figure 1.** A flow diagram of current approaches to isolate, concentrate and detect norovirus from shellfish.

The recovery rate of the elution and concentration approach has been reported to be highly variable (20–70 reverse transcription polymerase chain reaction unit (RT-PCR U)) within or between the protocols depending on the shellfish type and volume of shellfish digestive tissue [22–30] (Table 1). In addition, extraction processes can be time-consuming (requiring 1 to 3 days to process a batch...
of samples) [27]. An overview of the reported limit of detection (LOD) or recovery rate (%) of each method is presented in Table 1.

An alternative method to recover norovirus (and hepatitis A virus) from shellfish was developed that utilized the proteinase K enzyme to release the viruses from shellfish digestive tissue [22,35]. This method is simple, can be completed in a few hours and does not include a virus concentration step. The proteinase K digestion method has been extensively evaluated [20,30–32] and was included in the ISO 15216 standard method for the quantitative detection of norovirus and hepatitis A virus in 2017 [32]. The recovery rate of this method is reported to be higher (21 ± 15% to 34 ± 5%) than elution and concentration methods [20] (Table 1). An evaluation of the performance of the ISO 15216 method for the recovery and detection of norovirus and hepatitis A viruses in shellfish found that the limit of detection of norovirus (34 genome copies (GC)/g for norovirus GI and 53 GC/g for norovirus GII) was lower than for hepatitis A virus (198 GC/g) [36] (Table 1).

Despite reducing the time for sample preparation, the proteinase K digestion method has the potential to inactivate norovirus during the recovery process as the enzyme activity relies on a 65 °C heat treatment [37]. Therefore, this approach may not be suitable for the downstream determination of virus infectivity, which is essential when trying to measure the potential public health risk associated with shellfish consumption. As such, further research should focus on improving the recovery efficiency of norovirus from shellfish with minimal impact on infectivity.

Table 1. Limit of detection or recovery rates (%) of viruses from shellfish using various methods.

| Methods                        | Concentration Method (Where Applicable) | Shellfish (Weight) | Viruses | Limit of Detection/Recovery Rate | References |
|--------------------------------|----------------------------------------|-------------------|---------|----------------------------------|------------|
| Elution and concentration      | PEG                                    | Oysters (25 g)    | Norovirus | 22 RT-PCR U                     | [21]       |
|                                |                                        | Mussels (2 g)     | Norovirus GI | 20 RT-PCR U                  | [24]       |
|                                |                                        | Muscles (2 g)     | Rotavirus   | 10 RT-PCR U                 | [25]       |
|                                |                                        | Oysters (4 g)     | Rotavirus   | 1.39 GC/4 g                | [27]       |
|                                |                                        | Muscles (4 g)     | Rotavirus   | 1.39 GC/4 g                | [27]       |
|                                |                                        | Cockles (4 g)     | Rotavirus   | 1.39 GC/4 g                | [27]       |
|                                |                                        | Mussels (1.5 g)   | Mengovirus  | 1.8%                          | [28]       |
|                                |                                        | Oysters (1.5 g)   | Mengovirus  | 1.2%                          | [28]       |
|                                |                                        | Oysters (1.5 g)   | Norovirus GI | 70 RT PCR U/g                | [3]        |
|                                |                                        | Oysters (1.5 g)   | Norovirus GII | 70 RT PCR U/g          | [3]        |
|                                |                                        | Ultracentrifuge   | Oysters (25 g) | Hepatitis A virus | 9.9%       | [25]       |
|                                |                                        | Cationic separation | Oysters (5 g) | Hepatitis A virus | 20 RT-PCR U | [26]       |
| Proteinases K digestion        | Not applicable                         | Oysters (1.5 g)   | Norovirus GI | 20.5 ± 14.7%                | [20]       |
|                                |                                        | Mussels (25 g)    | Norovirus GI | 33.6 ± 5.3%                | [20]       |
|                                |                                        | Muscles (25 g)    | Norovirus GI | 3%                            | [30]       |
|                                |                                        | Oysters (3 g)     | Norovirus GI | 34 GC/g                      | [36]       |
|                                |                                        | Oysters (3 g)     | Norovirus GII | 53 GC/g              | [36]       |
|                                |                                        | Oysters (3 g)     | Hepatitis A virus | 198 GC/g            | [36]       |
| Direct RNA extraction          | Not applicable                         | Oysters (0.15 g)  | Norovirus   | 10 RT-PCR U                  | [34]       |
|                                |                                        | Oysters (5–50 g)  | Hepatitis A virus | 8 PFU                  | [38]       |

PEG, polyethylene glycol; PFU, plaque forming units; RT-PCR U, reverse transcription polymerase chain reaction unit; GC, genome copies; GI, genogroup I; GII, genogroup II.

Several studies have demonstrated the successful application of direct nucleic acid extraction from shellfish digestive tissue using zirconium beads [33,34,38]. The method detection limit was reported to be 10 RT-PCR U from 0.15 g of oyster digestive tissue [34] (Table 1). Although direct nucleic acid extraction methods are promising, unless combined with another approach, they are not able to discriminate between infectious and non-infectious norovirus. As such, they are not ideal for evaluating potential public health risks associated with shellfish consumption. In addition, direct nucleic acid extraction may not successfully remove PCR inhibitors present in the shellfish tissues that can hinder the downstream detection.
2. Current Norovirus Detection from Shellfish

Reverse transcription quantitative PCR (RT-qPCR) is widely used for the detection and quantification of norovirus from shellfish [3,18,22,32,39–41]. Unlike conventional RT-PCR, RT-qPCR utilizes fluorescently labelled probes that allow for the simultaneous confirmation of the presence of the specific target. For norovirus, RT-qPCR methods are rapid, sensitive and specific but as virus quantification depends on the use of a calibration standard curve, variability in quantification can occur between laboratories. The detailed RT-qPCR protocol with a description of the suite of controls required for quantification was described in the ISO 15216 method [32].

Digital PCR (dPCR), with a RT step (RT-dPCR) for norovirus, can overcome the requirement for a standard curve [42,43]. dPCR is an absolute end-point nucleic acid quantitative technique based on dividing the sample into many thousands of partitions, analyzing each partition by PCR and using Poisson statistics, rather than an external calibration curve, to quantitate. One dPCR approach is droplet digital PCR (ddPCR). In ddPCR, oil nanodroplets in water are first prepared and then subjected to qPCR analysis. The number of positive and negative nanodroplets after the qPCR assay is calculated and as for conventional dPCR, Poisson statistics are used for quantification. Another advantage of dPCR/ddPCR over qPCR is that it is reported to give more accurate quantification and is less prone to inhibitors that may be present in nucleic acid extracted from shellfish, even after purification. For samples with a high concentration of target, quantification may not be possible without dilution. For instance, one study using ddPCR reported 100% saturation of positive droplets at $10^{5}$ target copies per µL template [44].

Loop-mediated isothermal amplification (LAMP), with a RT step for norovirus, is another molecular method that has the potential to detect norovirus from shellfish faster, cheaper and with equal sensitivity to RT-qPCR [45,46]. The LAMP method uses auto-cycling strand displacement DNA synthesis under isothermal conditions. While RT-qPCR/RT-dPCR uses expensive specialized equipment such as thermal cyclers, LAMP only requires a waterbath or heat block to maintain the isothermal conditions, with product measured using a turbidity, coloration or fluorescence-based detector. This means that RT-LAMP has the potential to be used on-site [45,46]. Fukuda and colleagues combined nucleic acid sequence-based amplification (NASBA) with a RT-LAMP assay (NASBA-RT-LAMP) and evaluated the sensitivity against RT-semi-nested PCR. The sensitivity of the NASBA-RT-LAMP assay for detecting norovirus in oysters was reported to be equivalent to the RT-semi-nested PCR, being able to frequently detect less than 100 genome copies of norovirus in oysters [46].

Despite being rapid, sensitive and specific, molecular methods are unable to predict the infectivity of norovirus present in the shellfish. In recent years, RT-qPCR methods have been combined with a pretreatment such as enzymatic (RNase) [47–51], photoactivatable dyes (EMA, PMA, PMAxx and PEMAX) [52–56] and a platinum compound [57] for the selective detection of infectious norovirus. Other methods, such as porcine gastric mucin-binding [58–61] and in situ capture [62–67], have also been combined with RT-qPCR for this purpose. The working mechanism of modified RT-qPCR assays to determine infectivity is shown in Figure 2.

The applicability of these modified RT-qPCR assays is questionable due to their dependency on sample matrix, pathogen inactivation mechanism, treatment conditions, condition of binding site and lack of reproducibility [52,58,62,68]. As such, they may not provide an accurate estimation of infectivity. In addition, some studies have evaluated the applicability of these methods to determine the norovirus infectivity in shellfish and reported having limited success and inconsistent results [53,56]. These inconsistent results could be due to the use of different compounds or norovirus inactivation protocols such as heat, UV radiation and chlorination, which each damage norovirus by different mechanisms. For example, thermal inactivation can damage the norovirus capsid more effectively than UV radiation and chlorination. As a result, modified infectivity-based RT-qPCR assays may be more effective on thermally inactivated norovirus, and less applicable for UV radiation and chlorination treatments.
3. Emerging Detection Technologies

Due to the limitations of current methods including costs, lengthy times to obtain results and the necessity of advanced laboratory equipment, infrastructure and skilled personnel, there is a need for rapid and easy detection techniques for norovirus from shellfish with minimal manual sample handling. Early and accurate onsite detection and identification of norovirus contamination in shellfish tissue will reduce costs by: (a) eliminating sample transportation, (b) holding products in cold storage while routine testing is conducted, (c) minimizing operating, infrastructure, equipment and personnel costs linked to testing laboratories, and (d) limiting the impact caused due to farm closures or product recalls. These early and onsite pathogen detection technologies would not only reduce the risk of foodborne illness but also provide greater product assurance.

Recent advances in omics, nanotechnology, electrochemical and molecular detection technologies can improve the development of such rapid diagnostic devices [69–75]. In addition, advancements in
3D printing may improve the size and physical footprint of the detection devices and reduce their production cost [76].

3.1. Omics-Based Approaches

Oomics-based approaches such as metagenomics, proteomics and metabolomics have the potential to deliver rapid diagnostic techniques towards food safety research [69]. The use of omics-based approaches could result in a paradigm shift for food safety testing, as seen for human and veterinary medicine where there have been developments in rapid and precise pathogen detection and characterization [70]. Figure 3 illustrates the omics platforms that have the most potential for monitoring and assessing the presence of norovirus in shellfish.

![Omics Platforms](image)

**Figure 3.** Omics-based virus monitoring approaches.

### 3.1.1. Metagenomics

Metagenomics is the analysis of total nucleic acids, including those from viruses that are present in a complex biological matrix. Viral metagenomics offers an alternative approach to a pathogen-specific molecular method [77]. Metagenomics involves high-throughput sequencing of RNA/DNA amplicons from a matrix, generating a large amount of genomic data. Bioinformatic analysis of the metagenomics datasets then identify and characterize the microbial communities which provides additional information regarding viral genomes present [78,79]. Because other human enteric viruses can also be present in shellfish [80], metagenomics has the potential to detect multiple pathogens from a single sample reaction. For example, metagenomics was used to identify multiple viruses from an oyster-related acute gastroenteritis outbreak in Osaka City in Japan, which were attributed to other pathogens such as astrovirus, sapovirus and rotavirus [80].

Despite a lack of published studies performing untargeted viromes sequencing from shellfish samples, metagenomics can be beneficial for public health and for shellfish safety. With the recent development in the MinION platform (Oxford Nanopore Technologies), metagenomics-based methods have the potential to be used in the field as necessary. Currently, viral metagenomics is expensive and requires skilled personnel to analyze the information to produce a confirmative result. In addition, the sensitivity and specificity of the metagenomics approach has not been fully evaluated and is in need of more research.

### 3.1.2. Proteomics

Metaproteomics is the analysis of proteins produced by an organism or population of organisms, and their expression in the presence of pathogens and viruses. The proteome is defined as the entire...
set of proteins that are, or can be, produced by a genome and is different among individuals, cell types, or even within the same cell at different times or growth phases. Proteomics encompasses the scientific research of the proteome, including protein composition, structure, levels and unique activity patterns [81,82]. In recent years, proteomics-based matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) has emerged as a tool for pathogen identification and diagnostics from intact cells or cell extracts [81,83]. MALDI-ToF MS is a rapid and sensitive technique and has been used for food authentication [84] and the detection of foodborne and waterborne pathogens [85,86].

This technology primarily relies on the characterization of pathogens by analyzing the whole cell or viral proteome in a typical mass range \( m/z \) of 2–20 kDa [87]. One of the disadvantages of MALDI-ToF MS is that it is reliant on the existing spectral database of the mass fingerprints of the pathogen strains and is unable to identify new species of organisms. Proteomics can be complemented with other omics-based techniques such as metabolomics to develop a robust and reliable tool for pathogen identification and diagnostics. Similar approaches have been applied to assess food allergies and food safety in shellfish [88–90].

3.1.3. Metabolomics

Metabolomics is the analysis of the small chemical compounds (metabolites, molecular weight <1.5 kDa) produced and consumed by an organism or a population of organisms because of their environmental and genetic potential (including exposure to viruses). The metabolome comprises the final downstream product of the genome, transcriptome, and proteome, which reflects the phenotype of a biological system [82]. Traditionally, metabolomics has been extensively applied in disease diagnosis [91], agriculture [92,93] and toxicology [94].

Research around ‘food metabolomics’ has gained momentum in the last decade. Several studies have applied this approach for the detection of foodborne pathogens (e.g., *Listeria* spp.) [95–97]. A considerable proportion of metabolomic-based applications are focused on food composition and traceability of foods, food quality and food safety [98–100]. For example, Aru et al. [101] and Alfaro et al. [102] applied a metabolomics approach to analyze changes in the metabolic profile of mussels under various food storage conditions, correlating observed metabolite signatures with microbial counts as potential biomarkers of spoilage. Nguyen et al. [103] utilized metabolomics to assess the tissue-specific immune response of New Zealand Greenshell™ mussel (*Perna canaliculus*) infected with *Vibrio coralliilyticus*. Nguyen et al. [103] concluded that such an approach could be used to rapidly assess infected mussels by assessing the mussel immune response to infection. Others have utilized metabolomics of shellfish to assess climate change impacts and environmental contaminants [104,105].

Although there is great potential for omics-based approaches to detect norovirus, these methods are in the early stages of development and have not been evaluated extensively for the detection of norovirus from shellfish. Extraction of nucleic acid, metabolites, and protein may hinder its onsite application. Limitations of omics-based approaches need to be considered so that public health risks are not overestimated. Despite the limitations, omics-based approaches have the potential to provide or identify biomarkers that can be used to develop rapid onsite diagnostic sensors or kits for norovirus detection.

3.2. Emerging Technologies for Onsite Detection

3.2.1. Nanomaterials

Different nanomaterials, such as nanocrystals, quantum dots and graphene, are gaining interest as a potential agent for virus detection. For instance, functionalized rod-shaped, colloidal cellulose nanocrystals (CNCs) have been used for the detection of cowpea chlorotic mottle virus and norovirus virus-like particles (VLPs). Cationic polymer brush was generated on the surface of these CNCs to retain excellent dispensability and colloidal stability in water with the electrostatic binding of the
VLP [106]. The capture of norovirus VLPs by modified CNCs was verified by size (dynamic light scattering measurement) and electron microscopy.

Quantum dots (QDs) are small fluorescent labels made with CdSe-ZnS with unique emission properties on a single wavelength excitation. QDs were utilized on a Surface Plasmon Resonance (SPR) assisted immunoassay to detect norovirus VLPs [73]. Combining the SPR enhancement, intensity of auto-fluorescence, and excitation efficiency of quantum dots, the single-to-noise ratio was optimized to increase the sensitivity of the sandwich assay for the detection of norovirus VLPs from phosphate buffered saline (PBS). The newly developed assay was able to detect 100 VLPs from the PBS solution [73]. Another plasmonic sensor was developed by Junesch and colleagues, where the lipid bilayer membrane was developed for binding norovirus, enabling label-free and real-time detection [107]. The interaction of norovirus with glycosphingolipids induced negative membrane curvature or invaginations due to viral accumulation. This novel location specific sensor is superior to conventional SPR or to other planar detection surfaces and requires only an ordinary spectrophotometer in virus detection.

Novel nanostructure using a hybrid of graphene and gold is also being explored for the detection of norovirus VLPs [108]. Antibody conjugated graphene-gold nanoparticles catalyze the substrate to generate a visible blue color which is directly proportional to the concentration of the target. This nanostructure combined the enhanced Raman intensity and peroxidase-like catalytic activity of graphene and gold. This combinational approach allowed the assay to be 100 times more sensitive than conventional ELISA methods and detected 100 pg/mL of the target.

Most of the nanostructures like nanoparticles are utilized in combination with Lateral Flow Assays (LFA) for onsite detection of target pathogens [75]. Traditional LFA has limited sensitivity due to the background signal and lower signal intensity of commonly-used gold or blue latex nanoparticles. Phase nanoparticles have been used as a reporter based on antibodies identified from sandwich ELISAs. Validation of the LFA was performed using both gold and phase nanoparticles and showed phage nanoparticle LFA had a 100-fold lower LOD than the gold nanoparticle LFA using the same antibody pair.

3.2.2. Aptamer

Aptamers are short DNA, RNA and peptide-based sequences selected through systematic ligand evolution by an exponential enrichment (SELEX) process, which binds with the target (e.g., norovirus) based on its protein structure (Figure 4) [109–111]. Escudero-Abarca and colleagues developed four ssDNA aptamer candidates that targeted norovirus GII.4 but showed affinity to both GII.2 and GII.4 strains using an enzyme-linked aptamer sorbant assay [112]. The binding capacity of the aptamer was 13-14 VLPs, equivalent to that of a commercial anti-norovirus antibody (1 to 5 µg/mL). One of the four potential aptamer candidates (aptamer 25) developed by Escudero-Abarca and colleagues was coupled with a magnetic capture method for the detection of norovirus from artificially-contaminated lettuce. The capture efficiency of the magnetic capture method was 2.5 to 36% with a LOD of 10 RNA copies/lettuce sample [112].

To advance the aptamer-based detection technology, Moore and colleagues developed an aptamer-based technique not only to detect norovirus but also to demonstrate the confirmation-based binding. The aptamer was designed to target the P-domain protein of a norovirus GII.4 strain using E. coli to express and purify the P protein [109]. After SELEX, an aptamer named M6-2 was selected and confirmed for targeting norovirus GL7, GII.2, GII.4 and GII.7 strains with low to moderate binding affinity. Magnetic particle-based capture and RT-PCR demonstrated a LOD of 4.88 log_{10} input genome copies (GC). These aptamers could also be used in combination with multiple sensing platforms for the detection of murine norovirus (used as a norovirus surrogate) or norovirus. One such example is the work by Wang and colleagues, where they combined aptamers with Micro-Electro-Mechanical Systems (MEMS) to develop a biosensor for norovirus [113].
3.2.3. Biosensor-Based Detection

A biosensor is an object that transduces biological signals to measurable optical, electrical or physical signals [114]. The output signals are either displayed, stored or analyzed to generate useful diagnostic information [115]. A biosensor generally possesses an antibody/antigen, enzyme, nucleic acid, phage, cell, or biomimetic membrane as a receptor or signal transducer. The most commonly used bioreceptors are antibodies, and nucleic acids such as aptamers. Figure 5 illustrates the mechanism of sensor-based detection technologies for monitoring and assessing the presence of norovirus in shellfish.

Figure 4. A flow diagram showing an aptamer-based norovirus detection approach.

A miniaturized and portable MEMS-based electrochemical aptasensor was developed and evaluated for the detection of norovirus [113]. The electrode surface was functionalized with virus-specific fluorescent aptamers using drop-casting methods. The binding capability between the aptamer and the sensing electrode was evaluated by testing the sensor responses to different titers of murine norovirus. The MEMS aptasensor exhibited a rapid and clear response to different virus titers with a LOD of 50 plaque forming units (PFU)/mL.

Another electrochemical biosensor using Concanavalin A (ConA) conjugated with nanostructured gold electrode was developed to capture norovirus from food material within an hour with better specificity and sensitivity [116]. The study also demonstrated a LOD of norovirus from lettuce extract was 60 GC/mL with a specificity of 98% from a mixture containing hepatitis A virus, hepatitis E virus and norovirus.

A label-free homogeneous assay was developed using a split G-quadruplex nano-tweezer to detect a partial norovirus RNA [117]. The nano-tweezer, with a single signal-transducing molecule, could self-assemble from three single-stranded DNA molecules by simple mixing. Upon recognition of norovirus RNA, the signal molecule structure changed and restored its activity hence producing the detectable signal. The LOD was reported at 4 nM.
Multiple other biosensor assemblies have been published for potential norovirus detection from various sample sources [73,107,118]. For example, a thioglycolic-capped CdZnSeS quantum dot probe was developed for the detection of norovirus RNA with a high photo-luminescence quantum [118]. The sensitivity of this technology was reported to be 8.2 viral copies/mL with a specificity of 98%. Similarly, a gold-immobilized cysteine-incorporated peptide-based electrochemical biosensor was also evaluated for the detection of norovirus [119]. The reported LOD of this method was 7.8 copies/mL PBS. The described proof-of-concept studies showed potential for application to miniaturized micro-devices as a diagnostic tool for onsite detection of norovirus or other enteric viruses from shellfish.

3.2.4. Microfluidic Technology

Microfluidic technology is the miniaturization of molecular assays, which improves analytical performance by decreasing the consumption of reagents, detection time and human errors; while increasing sensitivity, reliability and the ability to detect multiple species of pathogens by integrating all necessary steps onto a single handheld disposable device [115,120]. Based on these advantages, microfluidic techniques offer promise for the rapid detection of norovirus from shellfish. During the assay, the sample passes through different regions on the microfluidics device either by capillary action or by pressure-driven by pumps. Multiple reactions of virus capture, isolation and identification can occur in different sections of microfluidic devices inside a closed system. Assays in closed systems ensures automated control of all steps and can reduce human errors and increase accuracy, reproducibility, and reliability of test results (Figure 6). Currently, there are two types of microfluidic technologies available for the detection of norovirus, (a) micro total analysis systems (lab-on-a-chip (LOC)) and, (b) paper-based analytical systems.

![Flow diagram of the lateral flow assay (LFA) detection method.](image)

Figure 6. Flow diagram of the lateral flow assay (LFA) detection method.

Typically, the LOC system is a silicon, glass, and polymer-based chip where all of the processes for detection can be completed and results can be obtained within a short period of time [75,121–123]. In addition to the advantages of conventional microfluidics, such as size, speed and reduced sample amount, paper-based LOC also adds an inexpensive multiplexed setting [124]. Paper is considerably easy to source, cheap, biodegradable and, most importantly, easy to modify chemically. Other advantages of paper devices include requiring no external power sources, a high ratio of surface-to-volume and minimal technical expertise requirements. Paper-based microfluidics, compared to microfluidics with LOC formats, has significant advantages such as it is a simpler technology and has reduced costs. However, paper-based microfluidics has issues in sample retention and evaporation that makes it less suitable for the detection of a low concentration of pathogens in a particular sample.

LOC modules have been used to detect murine norovirus with drop-based microfluidics [125]. A microfluidic platform combined with RT-dPCR was developed to amplify, detect and characterize genetic recombination between two murine norovirus strains. Another LOC-based norovirus detection method incorporated micro-bead beating to capture the virus and later this was used to lyse the virus inside the closed system [123]. This was achieved by switching the surface charge of the nanoparticles. An isothermal RNA amplification method and NASBA was utilized to detect murine norovirus from artificially contaminated oysters within 4 h. Viral RNA amplification and subsequent
detection was achieved with LOD of 100 PFU of murine norovirus per oyster. Another example of the application of LOC for norovirus detection in environmental and food samples is the use of microfluidic RT-qPCR. A microfluidic RT-qPCR has already been used for the simultaneous quantification of eleven major human viruses including enterovirus, Aichi virus, adenovirus, astrovirus, sapovirus, rotavirus, norovirus, hepatitis A virus and hepatitis E virus from environmental water samples [126]. High throughput quantitative information can be obtained with detection limits of 2 GC/µL of DNA or cDNA. The recent advancement on omics-based technology, nanomaterials, nanoenzymes, aptamers and biosensors could be utilized to develop more sensitive, cheap and rapid microfluidic devices in the near future.

In this review, we evaluated the advantages and disadvantages of the current detection methods and emerging technologies for the detection of norovirus from shellfish. Current nucleic acid-based detection methods including RT-qPCR, RT-dPCR and RT-LAMP/NASBA-RT-LAMP methods are rapid and sensitive and can be cheap (LAMP). However, these methods are unable to provide information on the infectivity of norovirus in shellfish. Molecular methods have been modified to inform on the infectivity of norovirus but still have limitations. In 2016, an in vitro cultivation method for norovirus using human intestinal enteroids was reported [127]. While the method is time consuming, expensive and still being optimized, the ability to culture norovirus will provide valuable infectivity information [128] and will enable better assessment of current detection methods to selectively detect infectious norovirus. Due to the limitations of current methods including cost, lengthy times to obtain results and the necessity of advanced laboratory equipment, infrastructure and skilled personnel, there is a need for rapid and easy detection techniques for norovirus from shellfish with minimal manual sample handling. Different rapid detection methods including aptamers, biosensors and microfluidic devices have been developed and evaluated for the rapid and sensitive detection of norovirus. So far, all emerging detection technologies have been tested using a simple matrix with a known concentration of norovirus. However, shellfish is a complex food matrix containing polysaccharides, glycogen and other compounds that may affect the efficacy of these emerging technologies. More research will be required to evaluate the performance of these new technologies in complex sample matrices such as shellfish. In addition, the continuous evolution of the norovirus RNA genome is another challenge that needs to be considered when developing onsite detection kits using emerging technologies. Despite the limitations, these technologies have the potential to be rapid and user-friendly detection kits that can be used for the detection of norovirus in real time.

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References
1. Hassard, F.; Sharp, J.H.; Taft, H.; LeVay, L.; Harris, J.P.; McDonald, J.E.; Tuson, K.; Wilson, J.; Jones, D.L.; Malham, S.K. Critical review on the public health impact of norovirus contamination in shellfish and the environment: A UK perspective. Food Environ. Virol. 2017, 9, 123–141. [CrossRef] [PubMed]
2. Teunis, P.F.M.; Moe, C.L.; Liu, P.; Miller, S.E.; Lindesmith, L.; Baric, R.S.; Le Pendu, J.; Calderon, R.L. Norwalk virus: How infectious is it? J. Med. Virol. 2008, 80, 1468–1476. [CrossRef] [PubMed]
3. Loisy, F.; Atmar, R.L.; Guillon, P.; Le Cann, P.; Pompeyuy, M.; Le Guyader, F.S. Real-time RT-PCR for norovirus screening in shellfish. J. Virol. Methods 2005, 123, 1–7. [CrossRef]
4. Le Guyader, F.S.; Atmar, R.L.; Le Pendu, J. Transmission of viruses through shellfish: When specific ligands come into play. Curr. Opin. Virol. 2012, 2, 103–110. [CrossRef]
5. Provost, K.; Dancho, B.A.; Ozbay, G.; Anderson, R.S.; Richards, G.P.; Kingsley, D.H. Hemocytes are sites of enteric virus persistence within oysters. *Appl. Environ. Microbiol.* **2011**, *77*, 8360–8369. [CrossRef]
6. Greening, G.E.; Hewitt, J.; Rivera-Aban, M.; Croucher, D. Molecular epidemiology of norovirus gastroenteritis outbreaks in New Zealand from 2002–2009. *J. Med. Virol.* **2012**, *84*, 1449–1458. [CrossRef] [PubMed]
7. Alfano-Sobsey, E.; Sweat, D.; Hall, A.; Breedlove, F.; Rodriguez, R.; Greene, S.; Pierce, A.; Sobsey, M.; Davies, M.; Ledford, S.L. Norovirus outbreak associated with undercooked oysters and secondary household transmission. *Epidemiol. Infect.* **2012**, *140*, 276–282. [CrossRef]
8. Bellou, M.; Kokkinos, P.; Vantarakis, A. Shellfish-borne viral outbreaks: A systematic review. *Food Environ. Virol.* **2013**, *5*, 13–23. [CrossRef]
9. Lodo, K.L.; Veitch, M.G.K.; Green, M.L. An outbreak of norovirus linked to oysters in Tasmania. *Commun. Dis. Intell. Q. Rep.* **2014**, *38*, 16–19.
10. Simmons, G.; Garbutt, C.; Hewitt, J.; Greening, G. A New Zealand outbreak of norovirus gastroenteritis linked to the consumption of imported raw Korean oysters. *N. Z. Med. J.* **2007**, *120*, U2773.
11. Le Guyader, F.S.; Le Saux, J.C.; Ambert-Balay, K.; Krol, J.; Serais, O.; Parnaudeau, S.; Giraudon, H.; Delmas, G.; Pompey, M.; Pothier, P. Aichivirus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *J. Clin. Microbiol.* **2008**, *46*, 4011–4017. [CrossRef] [PubMed]
12. Hall, A.J.; Lopman, B.A.; Payne, D.C.; Patel, M.M.; Gastañaduy, P.A.; Vinje, J.; Parashar, U.D. Norovirus disease in the United States. *Emerg. Infect. Dis.* **2013**, *19*, 1198–1205. [CrossRef] [PubMed]
13. Lim, K.L.; Hewitt, J.; Sitabkhan, A.; Eden, J.S.; Lun, J.; Levy, A.; Merif, J.; Smith, D.; Rawlinson, W.D.; White, P.A. A multi-site study of norovirus molecular epidemiology in Australia and New Zealand, 2013–2014. *PLoS ONE* **2016**, *11*, e0145254. [CrossRef] [PubMed]
14. Ahmed, S.M.; Hall, A.J.; Robinson, A.E.; Verhoef, L.; Premkumar, P.; Parashar, U.D.; Koopmans, M.; Lopman, B.A. Global prevalence of norovirus in cases of gastroenteritis: A systematic review and meta-analysis. *Lancet Infect. Dis.* **2014**, *14*, 725–730. [CrossRef]
15. Havelaar, A.H.; Kirk, M.D.; Torgerson, P.R.; Gibb, H.J.; Hald, T.; Lake, R.J.; Praet, N.; Bellinger, D.C.; De Silva, N.R.; Gargouri, N. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med.* **2015**, *12*, e1001923. [CrossRef] [PubMed]
16. Torgerson, P.R.; Devleesschauwer, B.; Praet, N.; Speybroeck, N.; Willingham, A.L.; Kasuga, F.; Koklinos, P.; Zhou, X.N.; Fèvre, E.M.; Sripa, B. World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: A data synthesis. *PLoS Med.* **2015**, *12*, e1001920. [CrossRef]
17. Costafreda, M.I.; Bosch, A.; Pinto, R.M. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. *Appl. Environ. Microbiol.* **2006**, *72*, 3846–3855. [CrossRef] [PubMed]
18. Le Guyader, F.S.; Bon, F.; DeMedici, D.; Parnaudeau, S.; Bertone, A.; Crudeli, S.; Doyle, A.; Zidane, M.; Suffredini, E.; Kohli, E.; et al. Detection of multiple noroviruses associated with an international gastroenteritis outbreak linked to oyster consumption. *J. Clin. Microbiol.* **2006**, *44*, 3878–3882. [CrossRef]
19. Nishida, T.; Nishio, O.; Kato, M.; Chuma, T.; Kato, H.; Iwata, H.; Kimura, H. Genotyping and quantitation of noroviruses in oysters from two distinct sea areas in Japan. *Microbiol. Immunol.* **2007**, *51*, 177–184. [CrossRef]
20. Le Guyader, F.; Parnaudeau, S.; Schaeffer, J.; Bosch, A.; Loisy, F.; Pompey, M.; Atmar, R.L. Detection and quantification of noroviruses in shellfish. *Appl. Environ. Microbiol.* **2009**, *75*, 618–624. [CrossRef]
21. Kingsley, D.H.; Richards, G.P. Rapid and efficient extraction method for reverse transcription-PCR detection of hepatitis A and Norwalk-like viruses in shellfish. *Appl. Environ. Microbiol.* **2001**, *67*, 4152–4157. [CrossRef] [PubMed]
22. Jothikumar, N.; Lowther, J.A.; Henshilwood, K.; Lees, D.N.; Hill, V.R.; Vinje, J. Rapid and sensitive detection of noroviruses by using TaqMan-based one-step reverse transcription-PCR assays and application to naturally contaminated shellfish samples. *Appl. Environ. Microbiol.* **2005**, *71*, 1870–1875. [CrossRef] [PubMed]
23. Casas, N.; Amarita, F.; de Maranon, I.M. Evaluation of an extracting method for the detection of Hepatitis A virus in shellfish by SYBR-Green real-time RT-PCR. *Int. J. Food Microbiol.* **2007**, *120*, 179–185. [CrossRef]
24. Baert, L.; Uyttendaele, M.; Debevere, J. Evaluation of two viral extraction methods for the detection of human noroviruses in shellfish with conventional and real-time reverse transcriptase PCR. *Lett. Appl. Microbiol.* **2007**, *44*, 106–111. [CrossRef]
25. Sincero, T.C.M.; Levin, D.B.; Simões, C.M.O.; Barardi, C.R.M. Detection of hepatitis A virus (HAV) in oysters (Crassostrea gigas). *Water Res.* 2006, 40, 895–902. [CrossRef]  
26. Papafragkou, E.; Plante, M.; Mattison, K.; Bidawid, S.; Karthikeyan, K.; Farber, J.M.; Jaykus, L.A. Rapid and sensitive detection of hepatitis A virus in representative food matrices. *J. Virol. Methods* 2008, 147, 177–187. [PubMed]  
27. Kittigul, L.; Singhaboot, Y.; Chavalitshewinkoon-Petmitr, P.; Pombubpa, K.; Hirunpetcharat, C. A comparison of virus concentration methods for molecular detection and characterization of rotavirus in bivalve shellfish species. *Food Microbiol.* 2015, 46, 161–167. [CrossRef]  
28. Uhrbrand, K.; Myrmel, M.; Maunula, L.; Vainio, K.; Trebbien, R.; Nærrung, B.; Schultz, A.C. Evaluation of a rapid method for recovery of norovirus and hepatitis A virus from oysters and blue mussels. *J. Virol. Methods* 2010, 169, 70–78. [CrossRef]  
29. David, S.T.; McIntyre, L.; MacDougall, L.; Kelly, D.; Liem, S.; Schallie, K.; McNabb, A.; Houde, A.; Mueller, P.; Ward, P.; et al. An outbreak of norovirus caused by consumption of oysters from geographically dispersed harvest sites, British Columbia, Canada, 2004. *Foodborne Pathog. Dis.* 2007, 4, 349–358. [CrossRef]  
30. Comelli, H.L.; Rimstad, E.; Larsen, S.; Myrmel, M. Detection of norovirus genotype L3b and II.4 in bioaccumulated blue mussels using different virus recovery methods. *Int. J. Food Microbiol.* 2008, 127, 53–59. [CrossRef]  
31. Lees, D. International standardisation of a method for detection of human pathogenic viruses in molluscan shellfish. *Food Environ. Virol.* 2010, 2, 146–155. [CrossRef]  
32. ISO. *Microbiology of the Food Chain—Horizontal Method for Determination of Hepatitis A Virus and Norovirus in Food Using Real-Time RT-PCR—Part-I: Method for Quantification; ISO 15216-1:2017; International Organization for Standardization: Geneva, Switzerland, 2017.*  
33. Boxman, I.L.A.; Tilburg, J.J.H.C.; Te Loeye, N.A.J.M.; Vennema, H.; Jonker, K.; de Boer, E.; Koopmans, M. Detection of noroviruses in shellfish in the Netherlands. *Int. J. Food Microbiol.* 2006, 108, 391–396. [CrossRef]  
34. De Roda Husman, A.M.; Lodder-Verschoor, F.; van den Berg, H.H.J.L.; Le Guyader, F.S.; Van Pelt, H.; van der Poel, W.H.M.; Rutjes, S.A. Rapid virus detection procedure for molecular tracing of shellfish associated with disease outbreaks. *J. Food Prot.* 2007, 70, 967–974. [CrossRef] [PubMed]  
35. Nuanualsuwan, S.; Cliver, D.O. Pre-treatment to avoid positive RT-PCR results with inactivated viruses. *J. Virol. Methods* 2002, 104, 217–225. [CrossRef]  
36. Lowther, J.A.; Bosch, A.; Butot, S.; Ollivier, J.; Made, D.; Rutjes, S.A.; Hardouin, J.; Lombard, B.; in’t Veld, P.; Leclercq, A. Validation of EN ISO method 15216-Part 1-quantification of hepatitis A virus and norovirus in food matrices. *Int. J. Food Microbiol.* 2019, 288, 82–90. [CrossRef] [PubMed]  
37. Langlet, J.; Kaas, L.; Croucher, D.; Hewitt, J. Effect of the shellfish proteinase K digestion method on norovirus capsid integrity. *Food Environ. Virol.* 2018, 10, 151–158. [CrossRef]  
38. Cromeans, T.L.; Nainan, O.V.; Margolis, H.S. Detection of hepatitis A virus RNA in oyster meat. *Appl. Environ. Microbiol.* 1997, 63, 2460–2463. [PubMed]  
39. Lowther, J.A.; Gustar, N.E.; Powell, A.L.; Hartnell, R.E.; Lees, D.N. Two-year systematic study to assess norovirus contamination in oysters from commercial harvesting areas in the United Kingdom. *Appl. Environ. Microbiol.* 2012, 78, 5812–5817. [CrossRef] [PubMed]  
40. Nishida, T.; Kimura, M.; Kozawa, K.; Kato, M.; Shinohara, M.; Fukuda, S.; Munemura, T.; Mikami, T.; Kwamoto, A.; et al. Detection quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. *Appl. Environ. Microbiol.* 2003, 69, 5782–5786. [CrossRef]  
41. Li, D.; Stals, A.; Tang, Q.J.; Uyttendaele, M. Detection of norovirus in shellfish and semi-processes fishery products from a Belgian seafood company. *J. Food Prot.* 2014, 77, 1342–1347. [CrossRef]  
42. Persson, S.; Eriksson, R.; Lowther, J.; Ellstrom, P.; Simonsson, M. Comparison between RT droplet digital PCR and RT real-time PCR for quantification of noroviruses in oysters. *Int. J. Food Microbiol.* 2018, 284, 73–83. [CrossRef] [PubMed]  
43. Polo, D.; Schaeffer, J.; Fournet, N.; Le Saux, J.C.; Parnaudeau, S.; McLeod, C.; Le Guyader, F.S. Digital PCR for quantifying norovirus in oysters implicated in outbreaks, France. *Emerg. Infect. Dis.* 2016, 22, 2189–2191. [CrossRef] [PubMed]  
44. Pinheiro, L.B.; Coleman, V.A.; Hindson, C.M.; Herrmann, J.; Hindson, B.J.; Bhat, S.; Emslie, K.R. Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. *Anal. Chem.* 2012, 84, 1003–1011. [CrossRef] [PubMed]
45. Jeon, S.B.; Seo, D.J.; Oh, H.; Kingsley, D.H.; Choi, C. Development of one-step reverse transcription loop-mediated isothermal amplification for norovirus detection in oysters. *Food Cont.* **2017**, *73*, 1002–1009. [CrossRef]

46. Fukuda, S.; Sasaki, Y.; Seno, M. Rapid and sensitive detection of norovirus genomes in oysters by a two-step isothermal amplification assay system combining nucleic acid sequences-based amplification and reverse transcription-loop-mediated isothermal amplification assays. *Appl. Environ. Microbiol.* **2008**, *74*, 3912–3914. [CrossRef] [PubMed]

47. Topping, J.R.; Schnerra, H.; Hainesa, J.; Scott, M.; Carter, M.J.; Willcocks, M.M.; Bellamy, K.; Brown, D.W.; Gray, J.J.; Gallimore, C.I.; et al. Temperature inactivation of feline calicivirus vaccine strain FCV F-9 in comparison with human noroviruses using an RNA exposure assay and reverse transcribed quantitative real-time polymerase chain reaction-A novel method for predicting virus infectivity. *J. Virol. Methods* **2009**, *156*, 89–95. [CrossRef]

48. Mormann, S.; Dabisch, M.; Becker, B. Effects of technological processes on the tenacity and inactivation of norovirus genogroup II in experimentally contaminated foods. *Appl. Environ. Microbiol.* **2010**, *76*, 536–545. [CrossRef]

49. Nowak, P.; Topping, J.R.; Fotheringham, V.; Gallimore, C.I.; Gray, J.J.; Iturriza-Gomara, M.; Knight, A.L. Measurement of the virolysis of Human GI.4 norovirus in response to disinfectants and sanitisers. *J. Virol. Methods* **2011**, *174*, 7–11. [CrossRef]

50. Diez-Valcarce, M.; Kovac, K.; Rasper, P.; Rodriguez-Lazaro, D.; Hernandez, M. Virus genome quantification does not predict norovirus infectivity after application of food inactivation processing technologies. *Food Environ. Virol.* **2011**, *3*, 141–146. [CrossRef]

51. Ronqvist, M.; Mikkela, A.; Tuominen, P.; Salo, S.; Maunula, L. Ultraviolet light inactivation of murine norovirus and human norovirus GI: PCR may overestimate the persistence of noroviruses even when combined with pre-PCR treatment. *Food Environ. Virol.* **2014**, *6*, 48–57. [CrossRef]

52. Gyawali, P.; Hewitt, J. Detection of infectious norovirus from wastewater and seawater using PEMAX™ treatment combined with RT-qPCR. *Water* **2018**, *10*, 841. [CrossRef]

53. Randazzo, W.; Khezri, M.; Ollivier, J.; Le Guyader, F.C.; Rodriguez-Diaz, J.; Aznar, R.; Sánchez, G. Optimization of PMAxx pretreatment to distinguish between human norovirus with intact and altered capsids in shellfish and sewage samples. *Int. J. Food Microbiol.* **2018**, *266*, 1–7. [CrossRef] [PubMed]

54. Karim, M.R.; Fout, G.S.; Johnson, C.H.; White, K.M.; Parshionikar, S.U. Propidium monoazide reverse transcriptase PCR and RT-qPCR for detecting infectious enterovirus and norovirus. *J. Virol. Methods* **2015**, *219*, 51–61. [CrossRef] [PubMed]

55. Leifels, M.; Jurzik, L.; Wilhelm, M.; Hamza, I.A. Use of ethidium monoazide and propidium monoazide to determine viral infectivity upon inactivation by heat, UV-exposure and chlorine. *Int. J. Hyg. Environ. Health* **2015**, *218*, 686–693. [CrossRef]

56. Li, X.; Huang, R.; Chen, H. Evaluation of assays to quantify infectious human norovirus for heat and high-pressure inactivation studies using Tulane virus. *Food Environ. Virol.* **2017**, *9*, 314–325. [CrossRef] [PubMed]

57. Fraisse, A.; Niveau, F.; Hennechart-Collette, C.; Coudray-Meunier, C.; Martin-Latil, S.; Perelle, S. Discrimination of infectious and heat-treated norovirus by combining platinum compounds and real-time RT-PCR. *Int. J. Food Microbiol.* **2018**, *269*, 64–74. [CrossRef]

58. Li, X.; Chen, H. Evaluation of the porcine gastric mucin binding assay for high pressure-inactivation studies using murine norovirus and Tulane virus. *Appl. Environ. Microbiol.* **2015**, *81*, 515–521. [CrossRef]

59. Dancho, B.A.; Chen, H.; Kingsley, D.H. Discrimination between infectious and non-infectious human norovirus using porcine gastric mucin. *Int. J. Food Microbiol.* **2012**, *155*, 222–226. [CrossRef]

60. Tian, P.; Engelbrektson, A.; Mandrell, R. Two-log increase in sensitivity for detection of norovirus in complex samples by concentration with porcine gastric mucin conjugated to magnetic beads. *Appl. Environ. Microbiol.* **2008**, *74*, 4271–4276. [CrossRef] [PubMed]

61. Li, D.; Baert, L.; Van Coillie, E.; Uyttendaele, M. Critical studies on binding-based RT-PCR detection of infectious noroviruses. *J. Virol. Methods* **2011**, *177*, 153–159. [CrossRef] [PubMed]
63. Wang, D.; Xu, S.; Yang, D.; Young, G.M.; Tian, P. New in situ capture quantitative (real-time) reverse transcription-PCR method as an alternative method for determining inactivation of Tulane virus. *Appl. Environ. Microbiol.* **2014**, *80*, 2120–2124.

64. Wang, D.; Tian, P. Inactivation conditions for human norovirus measured by an in situ capture-qRT-PCR method. *Int. J. Food Microbiol.* **2014**, *172*, 76–82. [CrossRef] [PubMed]

65. Cannon, J.L.; Vinje, J. Histo-blood group antigen assay for detecting noroviruses in water. *Appl. Environ. Microbiol.* **2008**, *74*, 6818–6819. [CrossRef]

66. Morton, V.; Jean, J.; Farber, J.; Mattison, K. Detection of norovirus in ready to eat foods using carbohydrate-coated magnetic beads. *Appl. Environ. Microbiol.* **2009**, *75*, 4641–4643. [CrossRef] [PubMed]

67. Zhou, Z.; Tian, Z.; Li, Q.; Tian, P.; Wu, Q.; Wang, D.; Shi, X. In situ capture RT-qPCR: A new simple and sensitive method to detect human norovirus in oysters. *Front. Microbiol.* **2017**, *8*, e554. [CrossRef]

68. Manuel, C.S.; Moore, M.D.; Jaykus, L.A. Predicting human norovirus infectivity—Recent advancement and continued challenges. *Food Microbiol.* **2018**, *76*, 337–345. [CrossRef]

69. Bergholz, T.M.; Moreno Switt, A.I.; Wiedmann, M. Omics approaches in food safety: Fulfilling the promise? *Trends Microbiol.* **2014**, *22*, 275–281. [CrossRef] [PubMed]

70. Ellis, D.I.; Muhamadali, H.; Chisanga, M.; Goodacre, R. Omics methods for the detection of foodborne pathogens. In *Encyclopedia of Food Chemistry*; Melton, L., Shahidi, F., Varelis, P., Eds.; Academic Press: Oxford, UK, 2019; pp. 364–370.

71. Liu, D.; Zhang, Z.; Yin, Y.; Jia, F.; Wu, Q.; Tian, P.; Wang, D. Development and evaluation of a novel in situ target-capture approach for aptamer selection of human noroviruses. *Talanta* **2019**, *193*, 199–205. [CrossRef]

72. Zarei, M. Advance in point of care technologies for molecular diagnostics. *Biosens. Bioelectron.* **2017**, *98*, 494–506. [CrossRef]

73. Ashiba, H.; Sugiyama, Y.; Wang, X.; Shirato, H.; Higo-Moriguchi, K.; Taniguchi, K.; Ohki, Y.; Fujimaki, M. Detection of norovirus virus-like particles using a surface plasmon resonance-assisted fluorimunosensor optimized for quantum dot fluorescent labels. *Biosens. Bioelectron.* **2017**, *93*, 260–266. [CrossRef] [PubMed]

74. Neethirajan, S.; Ahmed, S.R.; Chand, R.; Buozis, J.; Nagy, E. Recent advances in biosensor development for foodborne virus detection. *Nanotheranostics* **2017**, *1*, 272–295. [CrossRef] [PubMed]

75. Weng, W.; Neethirajan, S. Aptamer-based fluorometric determination of norovirus using a paper-based microfluidic device. *Microchim. Acta* **2017**, *184*, 4545–4552. [CrossRef]

76. Gong, H.; Woolley, A.T.; Nordin, G.P. 3D printed high density, reversible, chip to chip microfluidic interconnections. *Lab Chip* **2018**, *18*, 639–647. [CrossRef]

77. Nooij, S.; Schmitz, D.; Vennema, H.; Kroneman, A.; Koopmans, M.P.G. Overview of virus metagenomics classification methods and their biological applications. *Front. Microbiol.* **2018**, *9*, e749. [CrossRef] [PubMed]

78. Mokili, J.L.; Rohwer, F.; Dutilh, B.E. Metagenomics and future perspectives in virus discovery. *Curr. Opin. Virol.* **2012**, *2*, 63–77. [CrossRef]

79. Miller, R.R.; Montoya, V.; Gardy, J.L.; Patrick, D.M.; Tang, P. Metagenomics for pathogen detection in public health. *Genome Med.* **2013**, *5*, e81. [CrossRef]

80. Iritani, N.; Kaida, A.; Abe, N.; Kubo, H.; Sekiguchi, J.; Yamamoto, S.P.; Goto, K.; Tanaka, T.; Noda, M. Detection and genetic characterization of human enteric viruses in oyster-associated gastroenteritis outbreaks between 2001 and 2012 in Osaka City, Japan. *J. Med. Virol.* **2014**, *86*, 2019–2025. [CrossRef]

81. Fratamico, P.; Gunther, N.W. 24 Advances in genomics and proteomics-based methods for the study of foodborne bacterial pathogens. In *Advances in Microbial Food Safety*; Sofos, J., Ed.; Woodhead Publishing: Cambridge, UK, 2013; pp. 462–497.

82. Xu, Y.J. Foodomics: A novel approach for food microbiology. *TrAC Trends Analyt. Chem.* **2017**, *96*, 14–21. [CrossRef]

83. Singhal, V.; Kumar, M.; Kanaujia, P.K.; Virdi, J.S. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Front. Microbiol.* **2015**, *6*, e791. [CrossRef]

84. Ortea, I.; O’Connor, G.; Maquet, A. Review on proteomics for food authentication. *J. Proteomics* **2016**, *147*, 212–225. [CrossRef] [PubMed]

85. Vithanage, N.R.; Yeager, T.R.; Jadhav, S.R.; Palombo, E.A.; Datta, N. Comparison of identification systems for psychrotrophic bacteria isolated from raw bovine milk. *Int. J. Food Microbiol.* **2014**, *189*, 26–38. [CrossRef]
Foods 2019, 8, 187

86. Vithanage, N.R.; Bhongir, J.; Jadhav, S.R.; Ranadheera, C.S.; Palombo, E.A.; Yeager, T.R.; Datta, N. Species-level discrimination of psychrotrophic pathogenic and spoilage gram-negative raw milk isolates using a combined MALDI-TOF MS proteomics-bioinformatics-based approach. *J. Proteome Res.* 2017, 16, 2188–2203. [CrossRef]

87. Jadhav, S.; Gulati, V.; Fox, E.M.; Karpe, A.; Beale, D.J.; Sevior, D.; Bhave, M.; Palombo, E.A. Rapid identification and source-tracking of *Listeria monocytogenes* using MALDI-TOF mass spectrometry. *Int. J. Food Microbiol.* 2015, 202, 1–9. [CrossRef]

88. Villagómez-Márquez, R.; Montero, L.; Herrero, M. Application of mass spectrometry-based metabolomics for food safety, quality and traceability. *TrAC Trends Anal. Chem.* 2017, 93, 102–118. [CrossRef]

89. Nugraha, R.; Kamath, S.D.; Johnston, E.; Zenger, K.R.; Rolland, J.M.; O’Hehir, R.E.; Lopata, A.L. Rapid and comprehensive discovery of unreported shellfish allergens using large-scale transcriptomic and proteomic resources. *J. Allergy Clin. Immunol.* 2018, 141, 1501–1504. [CrossRef] [PubMed]

90. Ruethers, T.; Taki, A.C.; Johnston, E.B.; Nugraha, R.; Le, T.T.K.; Kalic, T.; McLean, T.R.; Kamath, S.D.; Lopata, A.L. Seafood allergy: A comprehensive review of fish and shellfish allergens. *Mol. Immunol.* 2018, 100, 28–57. [CrossRef]

91. Kouremenos, K.A.; Johansson, M.; Marriott, P.J. Advances in gas chromatographic methods for the identification of biomarkers in cancer. *J. Cancer* 2012, 3, 404–420. [CrossRef]

92. Hall, R.D.; de Maagd, R.A. Plant metabolomics is not ripe for environmental risk assessment. *Trends Biotechnol.* 2014, 32, 391–392. [CrossRef]

93. Lima, M.R.M.; Diaz, S.O.; Lamego, I.; Grusak, M.A.; Vasconcelos, M.W.; Gil, A.M. Nuclear magnetic resonance metabolomics of iron deficiency in soybean leaves. *J. Proteome Res.* 2014, 13, 3075–3087. [CrossRef]

94. Beale, D.J.; Morrison, P.D.; Palombo, E.A. Detection of listeria in milk using non-targeted metabolic profiling of *Listeria monocytogenes* 10403S grown at 37 °C and 8 °C. *Int. J. Food Microbiol.* 2011, 148, 107–114. [CrossRef]

95. Pinu, F.R. Early detection of food pathogens and food spoilage microorganisms: Application of metabolomics. *Trends Food Sci. Technol.* 2016, 54, 213–215. [CrossRef]

96. Castro-Puyana, M.; Pérez-Miguez, R.; Montero, L.; Herrero, M. Application of mass spectrometry-based metabolomics approaches for food safety, quality and traceability. *TrAC Trends Anal. Chem.* 2017, 93, 102–118. [CrossRef]

97. Singh, A.K.; Ulanov, A.V.; Li, Z.; Jayaswal, R.K.; Wilkinson, B.J. Metabolomes of the psychrotolerant bacterium *Listeria monocytogenes* 10403S grown at 37 °C and 8 °C. *Int. J. Food Microbiol.* 2011, 148, 107–114. [CrossRef]

98. Pinu, F.R. Metabolomics-The new frontier in food safety and quality research. *Food Res. Int.* 2015, 72, 80–81. [CrossRef]

99. Aro, V.; Pisano, M.B.; Savorani, F.; Engelsen, S.B.; Cosentino, S.; Cesare Marincola, F. Metabolomics analysis of shucked mussels’ freshness. *Food Chem.* 2016, 205, 58–65. [CrossRef]

100. Alfaro, A.C.; Nguyen, T.V.; Mellow, D. A metabolomics approach to assess the effect of storage conditions on metabolic processes of New Zealand surf clam (*Crasostrea aequilatera*). *Aquaculture* 2019, 498, 315–321. [CrossRef]

101. Aru, V.; Pisano, M.B.; Savorani, F.; Engelsen, S.B.; Cosentino, S.; Cesare Marincola, F. Metabolomics analysis of shucked mussels’ freshness. *Food Chem.* 2016, 205, 58–65. [CrossRef]

102. Alfaro, A.C.; Nguyen, T.V.; Young, T.; Merien, F. Tissue-specific immune responses to *Vibrio* sp. infection in mussels (*Perna canaliculus*): A metabolomics approach. *Aquaculture* 2019, 500, 118–125. [CrossRef]

103. Jadhav, S.; Gulati, V.; Fox, E.M.; Karpe, A.; Beale, D.J.; Sevior, D.; Bhave, M.; Palombo, E.A. Rapid identification and source-tracking of *Listeria monocytogenes* using MALDI-TOF mass spectrometry. *Int. J. Food Microbiol.* 2015, 202, 1–9. [CrossRef]

104. Eguchi, A.; Nomiyama, K.; Sakurai, K.; Kim Trang, P.T.; Viet, P.H.; Takahashi, S.; Iwata, H.; Tanabe, S.; Todaka, E.; Mori, C. Alterations in urinary metabolomic profiles due to lead exposure from a lead–acid battery recycling site. *Environ. Pollut.* 2018, 242, 98–105. [CrossRef]

105. Jiang, Z.; Wang, X.; Rastrick, S.P.S.; Fang, J.; Du, M.; Gao, Y.; Li, F.; Strand, Ø.; Fang, J. Metabolic responses to elevated pCO2 in the gills of the Pacific oyster (*Crassostrea gigas*) using a GC-TOF-MS-based metabolomics approach. *Comp. Biochem. Physiol.* 2019, 29, 330–338. [CrossRef] [PubMed]

106. Rosilo, H.; McKee, J.R.; Kontturi, E.; Koho, T.; Hytönen, V.P.; Ikkala, O.; Kostiainen, M.A. Cationic polymer brush-modified cellulose nanocrystals for high-affinity virus binding. *Nanoscale* 2014, 6, 11871–11881. [CrossRef] [PubMed]
107. Junesch, J.; Emilsson, G.; Xiong, K.; Kumar, S.; Sannomiya, T.; Pace, H.; Vörös, J.; Oh, S.H.; Bally, M.; Dahlin, A.B. Location-specific nanoplasmonic sensing of biomolecular binding to lipid membranes with negative curvature. *Nanoscale* 2015, 7, 15080–15085. [CrossRef] [PubMed]

108. Ahmed, S.R.; Takemeura, K.; Li, T.C.; Kitamoto, N.; Tanaka, T.; Suzuki, T.; Park, E.Y. Size-controlled preparation of peroxidase-like graphene-gold nanoparticle hybrids for the visible detection of norovirus-like particles. *Biosens. Bioelectron.* 2017, 87, 558–565. [CrossRef]

109. Moore, M.D.; Escudero-Abarca, B.I.; Suh, S.H.; Jaykus, L.A. Generation and characterization of nucleic acid aptamers targeting the capsid P domain of a human norovirus GII.4 strain. *J. Biotechnol.* 2015, 209, 41–49. [CrossRef] [PubMed]

110. Moore, M.D.; Bobay, B.G.; Mertens, B.; Jaykus, L.A. Human norovirus aptamer exhibits high degree of target conformation-dependent binding similar to that of receptors and discriminates particle functionality. *mSphere* 2016, 1, e00298. [CrossRef] [PubMed]

111. Giamberardino, A.; Labib, M.; Hassan, E.M.; Tetro, J.A.; Springthorpe, S.; Sattar, S.A.; Berezovski, M.V.; DeRosa, M.C. Ultrasensitive norovirus detection using DNA aptasensor technology. *PLoS ONE* 2013, 8, e79087. [CrossRef]

112. Escudero-Abarca, B.I.; Suh, S.H.; Moore, M.D.; Dwivedi, H.P.; Jaykus, L.A. Selection, characterization and application of nucleic acid aptamers for the capture and detection of human norovirus strains. *PLoS ONE* 2014, 9, e106805. [CrossRef]

113. Wang, N.; Kitajima, M.; Mani, K.; Kanhere, E.; Whittle, A.J.; Triantafyllou, M.S.; Miao, J. Miniaturized electrochemical sensor modified with aptamers for rapid norovirus detection. In Proceedings of the 11th Annual IEEE International Conference on Nano/Micro Engineered and Molecular Systems (IEEE-NEMS 2016), Matsushima Bay and Sendai MEMS City, Japan, 17–20 April 2016; pp. 587–590.

114. Marin, M.J.; Rashid, A.; Rejzek, M.; Fairhurst, S.A.; Wharton, S.A.; Martin, S.R.; McCauley, J.W.; Wileman, T.; Field, R.A.; Russell, D.A. Glyconanoparticles for the plasmonic detection and discrimination between human and avian influenza virus. *Org. Biomol. Chem.* 2013, 11, 7101–7107. [CrossRef]

115. Velusamy, V.; Arshak, K.; Korostynska, O.; Oliwa, K.; Adley, C. An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnol. Adv.* 2010, 28, 232–254. [CrossRef] [PubMed]

116. Hong, S.A.; Kwon, J.; Kim, D.; Yang, S. A rapid, sensitive and selective electrochemical biosensor with concanavalin A for the preemptive detection of norovirus. *Biosens. Bioelectron.* 2015, 64, 338–344. [CrossRef]

117. Nakatsuka, K.; Shigeto, H.; Kuroda, A.; Funabashi, H. A split G-quadruplex-based DNA nano-tweezers structure as a signal-transducing molecule for the homogeneous detection of specific nucleic acids. *Biosens. Bioelectron.* 2015, 74, 222–226. [CrossRef] [PubMed]

118. Adegoke, O.; Seo, M.W.; Kato, T.; Kawahito, S.; Park, E.Y. An ultrasensitive SiO2-encapsulated alloyed CdZnSeS quantum dot-molecular beacon nanobiosensor for norovirus. *Biosens. Bioelectron.* 2016, 86, 135–142. [CrossRef]

119. Hwang, H.J.; Ryu, M.Y.; Park, C.Y.; Ahn, J.; Park, H.G.; Choi, C.; Ha, S.D.; Park, T.J.; Park, J.P. High sensitivity and selective electrochemical biosensor: Label-free detection of human norovirus using affinity peptide as molecular binder. *Biosens. Bioelectron.* 2017, 87, 164–170. [CrossRef] [PubMed]

120. Gervais, L.; de Rooij, N.; Delamarche, E. Microfluidic chips for point-of-care immunodiagnostics. *Adv. Mater.* 2011, 23, H151–H176. [CrossRef] [PubMed]

121. Connelly, J.T.; Kondapalli, S.; Skoupi, M.; Parker, J.S.; Kirby, B.J.; Baemuner, A.J. Micro-total analysis system for virus detection: Microfluidic pre-concentration coupled to liposome-based detection. *Anal. Bioanal. Chem.* 2012, 402, 315–323. [CrossRef]

122. Tao, Y.; Rotem, A.; Zhang, H.; Chang, C.B.; Basu, A.; Kolawole, A.O.; Koehler, S.A.; Ren, Y.; Lin, J.S.; Pipas, J.M.; et al. Rapid, targeted and culture-free viral infectivity assay in drop-based microfluidics. *Lab Chip* 2015, 15, 3934–3940. [CrossRef] [PubMed]

123. Chung, S.H.; Baek, C.; Cong, V.T.; Min, J. The microfluidic chip module for the detection of murine norovirus in oysters using charge switchable micro-bead beating. *Biosens. Bioelectron.* 2015, 67, 625–633. [CrossRef] [PubMed]

124. Li, X.; Ballerini, D.R.; Shen, W. A perspective on paper-based microfluidics: Current status and future trends. *Biomicrofluidics* 2012, 6, 11301. [CrossRef]
125. Zhang, H.; Cockrell, S.K.; Kolawole, A.O.; Rotem, A.; Serohijos, A.W.; Chang, C.B.; Tao, Y.; Mehoke, T.S.; Han, Y.; Lin, J.S.; et al. Isolation and analysis of rare norovirus recombinants from coinfected mice using drop-based microfluidics. *J. Virol.* 2015, *89*, 7722–7734. [CrossRef]

126. Ishii, S.; Kitamura, G.; Segawa, T.; Kobayashi, A.; Miura, T.; Sano, D.; Okabe, S. Microfluidic quantitative PCR for simultaneous quantification of multiple viruses in environmental water samples. *Appl. Environ. Microbiol.* 2014, *80*, 7505–7511. [CrossRef] [PubMed]

127. Ettayebi, K.; Crawford, S.E.; Murakami, K.; Broughman, J.R.; Karandikar, U.; Tenge, V.R.; Neill, F.H.; Blutt, S.E.; Zeng, X.L.; Qu, L.; et al. Replication of human noroviruses in stem cell-derived human enteroids. *Science* 2016, *353*, 1387–1393. [CrossRef] [PubMed]

128. Costantini, V.; Morantz, E.K.; Browne, H.; Ettayebi, K.; Zeng, X.L.; Atmar, R.L.; Estes, M.K.; Vinje, J. Human norovirus replication in human intestinal enteroids as model to evaluate virus inactivation. *Emerg. Infect. Dis.* 2018, *24*, 1453–1464. [CrossRef] [PubMed]

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