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Current state-of-the-art diagnostics for Norovirus detection: Model approaches for point-of-care analysis

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ABSTRACT

Background: The implementation of fast, sensitive and specific diagnostic tools such as those for point-of-care testing (**POCT**) can assure public health security and **food safety** against foodborne infectious **disease outbreaks**. Norovirus is the most common pathogen causing foodborne outbreaks and the most frequent cause of acute gastroenteritis in humans. This frequency in outbreaks highlights the importance of the development and application of new portable diagnostic concepts and sensitive, multiplex, accurate, real-time (SMART) technologies considering the recommendations of Public Health Authorities accentuating personal and food hygiene measures to limit the spread of the virus. The development of POCT using handheld devices has significantly increased in recent years to detect norovirus due to the undeniable advantages of these methods such as rapidness, ultra-sensitivity and simplicity for norovirus diagnosis, in comparison to the 'gold standard' quantitative polymerase chain reaction (qPCR).

Scope and approach: Recent progress in the development of POCT devices and changing detection priorities in the food industry highlights the importance of efficient POCT methods selection, therefore, this review sought to outline the comparison of emerging POCT methods to be used for the analysis of food, water and clinical samples including molecular methods, biosensing technologies, immunodiagnostics, microarrays, and other prospective methods whereby implementation could aid the investigation of outbreaks of norovirus.

Key findings: In this study, advantages and disadvantages of POCT methods are illustrated and the prospective identification of POCT methods are compared and discussed. In summary, the concluding remarks illustrate the future trends and directions for POCT methods which can be applied to detect norovirus and other viruses.

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1. Introduction

Noroviruses are a major causative agent of foodborne gastrointestinal (GI) disease, estimated to be associated with 18% of all diarrhetic cases reported globally (Ahmed et al., 2014; Inns et al., 2017). Noroviruses are highly infectious viruses and GI disease may be caused by very low doses of norovirus virions (1–10 virion particles) (Teunis et al., 2008; Atmar, Ramani, & Estes, 2018). Therefore, infections caused by norovirus are widespread and frequently reported (Li et al., 2018). Global outbreaks occurring in the winter seasons caused by the genogroup II, genotype 4 (GII.4) variant are estimated to be in the region of 700 million cases leading to 220,000 deaths annually of which more than 70% of these cases are affecting children under five in developing countries (Africa and South East Asia) (Lane et al., 2019). The infection caused by norovirus is usually transmitted from person to person contact (i.e vomiting incidences) or contaminated food, water and surfaces (Gaythorpe et al., 2018) with mild characteristic symptoms such as vomiting and diarrhoea with a recovery period of 1–2 days (Inns et al., 2017). However, poorer outcomes are related with the elderly, immunocompromised and neonates' patients (Inns et al., 2017). In addition to

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the threat related to mortality caused by norovirus, in the UK alone the healthcare costs for the treatment of patients were estimated to reach £81 million annually (Tam & O'Brien, 2016).

Norwalk virus, first described in 1968 (Kapikian et al., 1972) following an outbreak of gastroenteritis in the town of Norwalk Ohio and named in 1972, is the prototype strain of a group of non-cultivatable, non-enveloped, positive sense single-stranded RNA viruses belonging to the genus *Norovirus* of the *Caliciviridae* family (Chhabra et al., 2019, Vinje et al., 2019). Noroviruses can be classified into 10 genogroups (GI-GX) based on the sequence diversity of their major structural capsid protein (VP1) which can be further divided into at least 48 genotypes known to cause infections in humans (Chhabra et al., 2019; International Committee on Taxonomy of Viruses, 2019). However, the genotypes GII.11, GII.18, GII.19, GIV.2, GIII, GV, GVI and GVII viruses are known to cause infections in different animal species (Vinje et al., 2019).

The past pandemic outbreaks of norovirus since 1968 (Karst & Baric, 2015) have led to a study of the genomic characterization of the virus variants causing infection, development of commonly available vaccines against the virus and evaluation of immunodiagnostic and molecular methods for the detection of outbreaks caused by GI disease causing noroviruses (Malik et al., 2019). Among these methods, only RT-qPCR has demonstrated proven sensitivity, and is the method recommended as the best practice in the detection of norovirus from food matrices such as shellfish (CEFAS 2020). These techniques, although sensitive have limitations (i.e lengthy procedures and the use of specialized equipment). Thus, there is still a requirement for rapid POCT devices such as biosensors or lateral flow immunoassays for norovirus detection. For the design and construction of a biosensor the key factors are sensitivity, specificity, reliability, cost-efficiency and the possibility of on-line use for environmental analysis but also simplicity in use for clinicians for clinical analysis and food operators for food analysis. Functional bio-recognition elements are the key components, which define the affinity (low detection limit), specificity (low interference), dynamic range, response time and lifetime of the biosensing system. Although, currently most of the developed biosensor-based methods are not able to compete with traditional methods in terms of precision or reproducibility, they offer the opportunity of continuous on-site and real-time monitoring of a contamination and may provide timely information about potential contamination (Kivirand & Rinken, 2019) (Fig. 1). Portable biosensing approaches in tandem with new bio-recognition elements are routinely being evaluated for enhanced sensitivity for virus detection and their suitability and use in the field.

Additionally, biosensors may be adopted for the multiplexing capabilities in the detection of several different species of genogroups within one species. This is particularly important as the diversity in the norovirus genomes and the antigenic drift of certain strains (e.g., GII.4) over time has posed limitations in single binding event measurements for biosensor development for its detection. In contrast, microarrays can detect multiple strains and are an attractive tool in development for the detection and analysis of noroviruses.

Therefore, the aim of this review is to compare the available POCT methods for noroviruses detection to enable robust and efficient diagnosis and when applied to food safety monitoring may be used to counterattack future NoV outbreaks. Finally, using the present literature on the topic and research experience, we would like to propose the future direction of POCT methods that in our view would prove useful, fast and efficient detection technologies and could replace current 'gold standard methods'.

2. POCT methods for detection of noroviruses

POCT methods have indisputable potential to provide sensitive and specific detection to manage patients immediately (i.e investigating transmission rate, protection against spread of the virus and fast GI disease treatment caused by norovirus) or to monitor for the virus in contaminated food and water to prevent the spread of infection. The methods compared in this review include molecular methods, biosensor

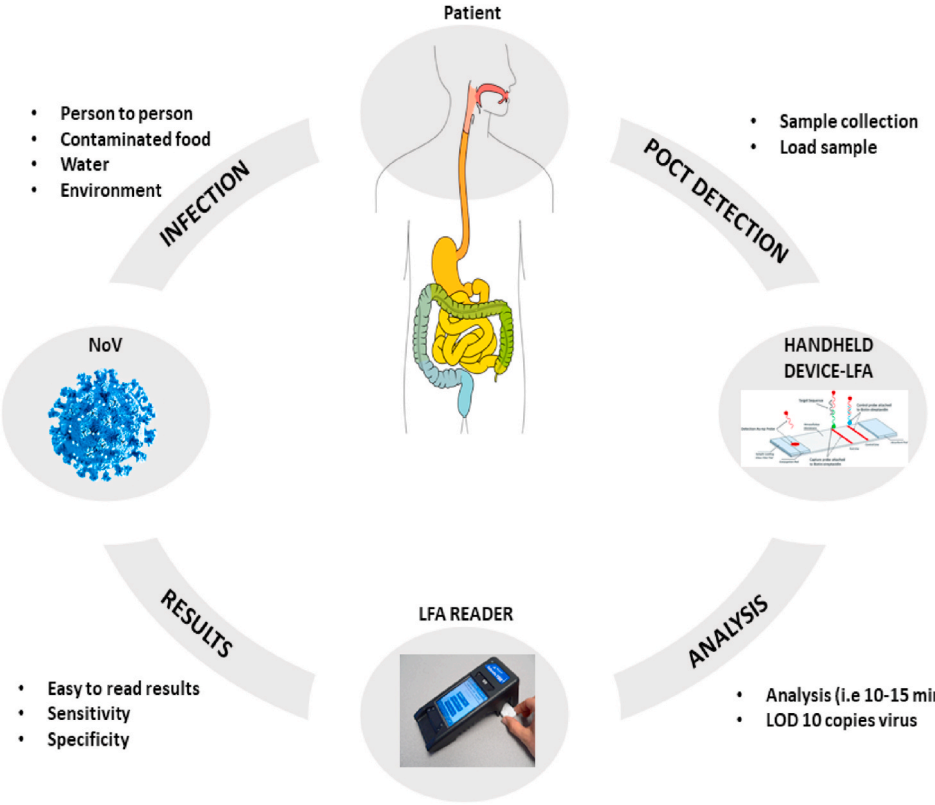

Fig. 1. Schematic process of sensitive and specific POCT detection of norovirus using a lateral flow handheld device.

based methods, immunoassays (EIA and LFA), microarrays and new perspectives on the conception of biosensor usage that could be implemented for the detection of noroviruses (Fig. 2).

2.1. Molecular methods

Over the last decade, significant progress has been made in the development of nucleic acid diagnostic methods for the routine detection of human noroviruses, with RT-qPCR assays set as the 'gold standard' for detection of noroviruses (Liu & Moore, 2020). The main disadvantages of the 'gold standard' RT-qPCR method are the lengthy procedure and difficulty in interpretation of positive results from asymptomatic individuals (Miura et al., 2018). Furthermore, the effect of inhibitory factors such as pH, particulates and ionic strength especially from food matrices cause more difficulties in interpretation (Bosch et al., 2018). The need for an alternative molecular method that can be used for on-site settings as a routine rapid test led to the development of isothermal amplification or portable real-time sequencing devices (Martzy et al., 2019; ONT 2020), each with their advantages and disadvantages as herein reviewed.

2.1.1. Isothermal amplification

The amplification of the specific nucleic acid sequences represents a crucial step for the development of sensitive detection systems. In comparison to standardized methods such as RT-qPCR or RT-qPCR/RT-dPCR, which are expensive and time-consuming (ISO 2019), isothermal amplification methods can provide a rapid, less expensive on-site diagnosis, with the results visualised by the naked eye (Gyawali et al., 2019). Isothermal methods, that operate at constant temperature, have emerged as promising alternatives to PCR and greatly simplify the implementation of amplification methods in point-of-care diagnostic devices and devices to be used in resource-limited settings. A review of the different isothermal amplification methods for nucleic acid detection and their integration into microfluidic devices has been previously conducted (Giuffrida & Spoto, 2017). These methods include loop-mediated isothermal amplification (LAMP), reverse transcription (RT) LAMP, recombinase polymerase amplification (RPA), nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), linear isothermal amplification methods such as rolling circle (RCA) and strand displacement (SDA). Isothermal methods stand out for their specificity, sensitivity and simplicity with the ability to detect a small number of copies of virus/reaction within a relatively short time (Martzy et al., 2019). Thus far, several sensitive isothermal methods have been reported detecting low levels of norovirus present in food or clinical samples at approximately 10–1000 copies virus/reaction includingNASBA, RPA and (RT-LAMP) (Table 1). NASBA, introduced in 1991, was designed to amplify single-stranded RNA targets (Compton, 1991). It operates at 41 °C and uses three enzymes (i.e., reverse transcriptase, RNase H and T7 DNA dependent RNA polymerase) and two primers (forward P1 and reverse P2) to mimic the *in vivo* retroviral replication mechanisms to produce RNA amplicons from an RNA template. RPA, introduced in 2006 (Piepenburg et al., 2006), was the construct of a new probe-based amplification approach to PCR exploiting the specific action of recombinase. RPA takes advantage of its simplicity, flexibility, low operating temperature (about 37 °C) and speed (results 5–20 min) (Daher et al., 2016). LAMP, introduced in 2000, is a single-tube technique for the amplification of DNA and RT-LAMP combines LAMP with a reverse transcription step to allow the detection of RNA (Notomi et al., 2000; Safavieh et al., 2016). However, a vast number of these methods although evaluated for their integration into microfluidic devices with the ambition to develop POCT have neither been optimized as simple colorimetric POCT methods or their efficacy tested using real-time qPCR. Isothermal amplification might be a promising efficient diagnostics tool for norovirus disease outbreaks, in comparison to the traditional 'gold standard' method (Malik et al., 2019). Table 1 highlights the assays developed as POCT methods for the detection of norovirus from faecal, water and animal samples and compares their detection limit, matrices tested and assay time. However, out of all these isothermal amplification assays (Table 1) (Fukuda et al, 2006, 2007, 2008; Greene et al., 2003; Houde et al., 2006; Moore et al., 2004Moore & Jaykus, 2017; Patterson et al., 2006; Yoda et al., 2007), LAMP colorimetric-based visualisations are the most promising POCT methods that enable the user to perform the test as a single-tube reaction (Luo et al., 2014). Luo and co-authors developed a hydroxynaphtol blue (HNB)-based LAMP assay targeting RNA-dependent RNA polymerase and the capsid protein gene of norovirus GII., showing high specificity to the target and sensitivity to detect 10^3 copies virus/reaction (Luo et al., 2014). Moreover, this assay when tested on clinical specimens showed 94.83% coincidence rate for norovirus GII. detection compared to RT-PCR (Luo et al., 2014). Of the several isothermal amplification

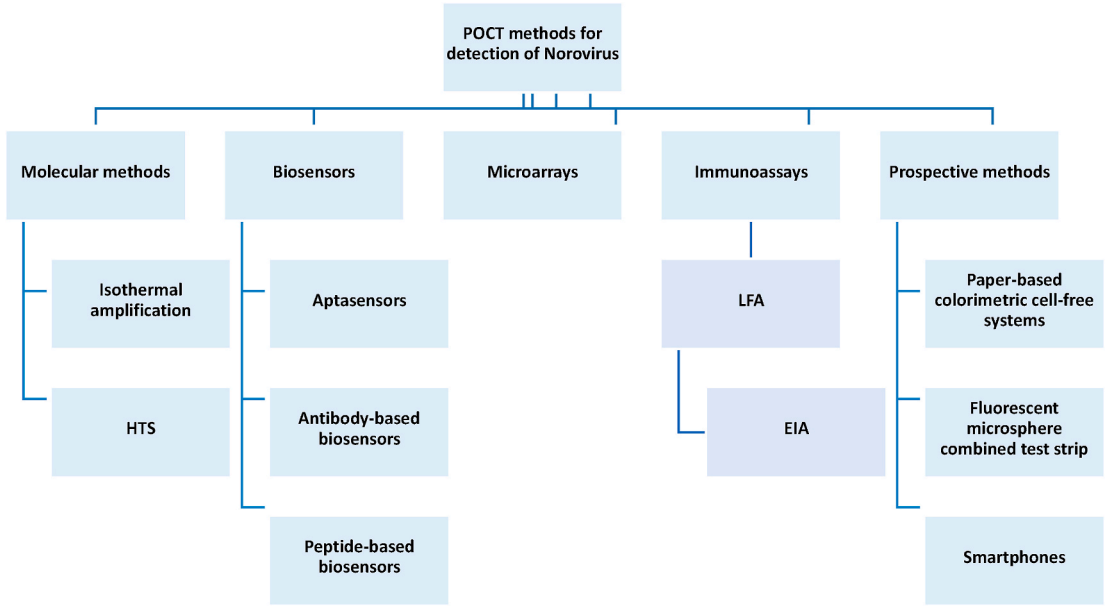

Fig. 2. A schematic representation of POCT diagnostic methods for Norovirus. Presented diagnostic assays are classified into five major categorizes: a) molecular methods, b) biosensors, c) microarrays, d) immunoassays, e) prospective methods, which have further sub-classifications.

Table 1
Developed assays as POCT methods for detection of Norovirus (NoV) from faecal, water and animal samples.

Detection method	Virus	Detection limit	Matrix	Assay time	References
Multiplex RT-NASBA	Rota virus NoV GII Astrovirus	7, 100, 200 copies/reaction respectively	Fecal samples	10–30 min	Mo et al. (2015)
Microfluidic chip combined with NASBA	Murine NoV	10^{2} PFU/single oyster	Spiked Oyster	4 h	Chung et al. (2015)
Molecular beacon based RT-NASBA	NoV GII	0.01 particles detectable units	Fecal samples	94 min	Lamhoujeb et al. (2009)
NASBA-RT-LAMP	NoV GI/4, GII/4	N/A	Oyster	3 h	(Fukuda et al., 2008; Houde et al., 2006)
NASBA combined with dot blot hybridization	NoV GII	N/A	Fecal samples	N/A	Houde et al. (2006)
NASBA combined with Agarose gel and dot blotting	NoV GI, GII	5 pg/ml 100 pg/1.5 g shellfish tissue	Fecal samples Spiked shellfish	N/A	Kou et al. (2006)
Molecular beacon based RT-NASBA	NoV GII	10-100 copies	Fecal samples	<1h	Patterson et al. (2006)
Multiplex NASBA combined with Agarose gel electrophoresis and dot blotting	Hepatitis A NoV GI, GII	10-100 PFU/9 cm	Spiked deli sliced turkey and lettuce	N/A	Jean et al. (2004)
Detection method	**Virus**	**Detection limit**	**Matrix**	**Assay time**	**References**
Commercial NASBA (NucliSens Basic Kit)	NoV GI, GII	10^{4} PFU	Fecal samples	4–6 h	Fukuda et al. (2008)
RT-LAMP	NoV 22	copies/μL	stream water	40min	Khairuddin et al. (2017)
RT-LAMP	NoV GI, GII	10 genome copies/μl	Feces/Spiked Oyster	NA	Jeon et al. (2017)
RT-LAMP	NoV GII	10^{3} copies/reaction	Fecal samples	1 h	Luo et al. (2014)
RT-LAMP	NoV GI, GII	Sensitivity range of 200-$8x10^{4}$ copies/sample	Fecal samples	N/A	Yoda et al. (2007)
RT-LAMP	NoV GI, GII	10^{2}-10^{3} copies/tube	Fecal samples	60–90 min	Fukuda et al. (2006)
RT-RPA	NoV GII.4	0.20 log10 copies	Fecal samples	30min	(Moore & Jaykus, 2017)
Split Gq based DNA-TN	NoV GII. Partial mRNA capsid	4 nM	Fecal samples	N/A	Nakatsuka et al. (2015)
Aptasensor	Murine NoV, GII.3	180 virus particle	N/A	60min	Giamberardino et al. (2013)
MEMS-based electrochemical aptasensor	MNV	N/A	Environment	N/A	Kitajima et al. (2016)
Detection methods	**Virus**	**Detection limit**	**Matrix**	**Assay time**	**References**
'Non-stop' aptasensor	NoV GII.	80 ng/mL of NoV DNA	Water/urine samples	30min	Kim et al. (2018)
NanoZyme aptasensor	MNV	20 copies virus/reaction	Fecal samples	10min	(Weerathunge, 2019)
V-trench antibody-based biosensor	NoV GII.4	100 copies/mL	N/A	30min	Ashiba et al. (2017)
'Concanavalin A' antibody-based biosensor	NoV GII.4	35-60 copies/mL	N/A	N/A	Hong et al. (2015)
LPFGs-immunosensor	NoV	1 ng/mL	N/A	40min	Janczuk-Richter et al. (2020)
Noro-1' peptide-based biosensor	NoV GII.4	7.8 copies/mL	Fecal	30min	Hwang et al. (2017)
peptide-based plasmonic biosensor	NoV	9.9 copies/mL	N/A	N/A	(Heo et al., 2019)
NoroBP-nonFoul (FlexL) peptide-based biosensor	NoV	1.7 copies/mL	Oysters	30min	Baek et al. (2019)
Tilling microarray	NoV	250-500 virus particle 10^{4} viral genome	Food	N/A	Yu et al. (2016)
Multiplex LLMDA assay	NoV Rotavirus Enterobacterias	N/A	N/A	N/A	Thissen et al. (2014)
Detection method	**Virus**	**Detection limit**	**Matrix**	**Assay time**	**References**
NoroChip V2.0 and V3.0	NoV GI.I/GII.4	N/A	N/A	N/A	Pagotto et al. (2008a, 2008b)
Generic microarray	NoV GI and GII	N/A	N/A	N/A	Jaaskelainen et al. (2006)

N/A-not applicable.

methods, LAMP assays involved 6–8 primers with loop formation which enhance its sensitivity and specificity to the selected target sequence (Becherer et al., 2020). However, the disadvantage of this method remains as the relatively high temperature of amplification (60–65 °C) in comparison to for exampleRPA (approx. 30–37 °C). Nonetheless, the future outlook is that the highly sensitive detection of nucleic acids based on digital LAMP may also be implemented into portable devices using a camera phone as a visual readout for the nucleic acid detection (Rodriguez-Manzano et al., 2016). In this case the smartphone camera can be exploited for the detection of a colorimetric assay while LAMP isothermal amplification is performed in wells of 5 nL in volume. However, integrating the isothermal amplification in POC structures that replicate the entire workflow for sample treatment to nucleic acid sequences detection requires further technological innovations.

2.1.2. High throughput sequencing (HTS)

Introduction of HTS sequencing platforms such as Illumnia or sequencing by oligonucleotide ligation and detection (SOLiD) have revolutionized the detection sector and these have become state-of-the-art techniques that enable precise detection and classification of microbes and viruses present in food and clinical samples, however the costs of analysis is still relatively high in comparison to other methods such as RT-PCR (Sekse et al., 2017). Within well introduced HTS methods such as next-generation sequencing (NGS) that has been implemented in multiple sectors, new third-generation sequencing (TGS) platforms (i.e Oxford Nanopore or Pacbio Sequel) are growing in popularity due to their speed with a relatively short time requirement for a library preparation (i. e Oxford Nanopore Technologies (ONT) MinION 10–60 min) and shorter length of analysis (3–24 h) (Theuns et al., 2018) in comparison to NGS (i.e library preparation for Illumnia platform up to 3 days and length of analysis approx. 56 h) (Head et al., 2014). The MinION sequencer ONT is a real-time sequencing portable nanopore microarray device built on membranes in a single flow cell, where DNA/RNA molecules are loaded onto the flow cell, following migration through nanopores (Grädel et al., 2019). This device has recently been reported to be a sufficiently fast on-site method for

recovering at least 85% of norovirus G.II genomes with relatively lower but enough accuracy in comparison to PacBio Sequel (Li et al., 2020). Despite the simplicity to perform TGS analysis using a MinION, high errors of base-calling and a lower coverage than the PacBio Sequel are disadvantages of this miniaturised device (Li et al., 2020). Another disadvantage of this device is the amount of input DNA/RNA required to perform sequencing (i.e 400 ng or 1–5 ng for barcoding kits) in comparison to the Illumnia platform which can obtain the whole genome with an input of only 0.2 ng of DNA/RNA.

2.2. Biosensors

Recently, the development of norovirus biosensors has risen significantly indicating their increased applications for norovirus disease detection (Ozer et al., 2020) with promising results reported in several studies with biosensors classified as simple, sensitive and specific analytical tools with the ability to rapidly analyse multiple samples compared to the conventional methods (Gyawali et al., 2019). Biosensors are analytical devices consisting of a recognition molecule, transducing element and detector. Current transducing elements are divided into major groups such as optical, electrochemical, thermometric, piezoelectric and magnetic. Biosensors produce an electronic or optical signal proportional to the specific interaction between the analyte and the recognition molecule such as antibodies, aptamers or single-stranded DNA which present on the sensor (Bhalla et al., 2016). A review of assays developed on biosensor devices is provided in Table 1. However, denaturation, non-specific binding to non-target molecules, sample preparation, system integration and sample matrix interference are continuously reported as obstacles for biosensor technology (Lichtenberg et al., 2019). Advances in nanotechnology and combining microfluidics, all provide advantages such as multiplexing, reducing sample size and ease of fluid control and process automation for biosensing (Liu & Moore, 2020). Additionally, the selection of biorecognition molecules (antibodies, aptamers and peptides) used for POCT is challenging for development with suitable specificity and selectivity of the sensing assay to detect noroviruses strains (Liu & Moore, 2020). In addition to the advantages, all of these have limitations which are highlighted in (Fig. 3).

2.2.1. Antibody-based biosensors

A selective electrochemical biosensor with concanavalin A has been developed for norovirus GII.4 detection (Hong et al., 2015). The developed electrochemical biosensor consisted of a nanostructured gold electrode conjugated with concanavalin (ConA); a lectin (carbohydrate-binding protein) originally extracted from jack beans (*Canavalia ensiformis*). This protein can selectively capture norovirus versus hepatitis A and hepatitis E (Hong et al., 2015). The developed biosensor could detect norovirus in the range of 10^2 to 10^6 copies/mL with a detection limit of in 35 and 60 copies/mL in buffer and spiked lettuce samples respectively (Hong et al., 2015). Furthermore, the selectivity of the biosensor tested against Hepatitis A and E was proven to have an approximately 98% selectivity with good reproducibility of RSD = 4.38% and thermal stability of 2.5%–3.1% decrement at 4 °C and 25 °C, respectively (Hong et al., 2015). In a study conducted by Ashiba and co-authors a simple V- shape trench biosensor was used for the detection of norovirus like particles GII.4 using surface plasmon resonance (SPR) (Ashiba et al., 2017). A sandwich immunoassay was applied using a monoclonal antibody as a capturing molecule and Qdot fluorescent dyes as a label on a surface of an Al film with the sensitivity of the assay reported to be 0.01 ng/mL, which was estimated to be equal to 100 virus-like particles. However, the cross reactivity of the biosensor wasn't tested (Ashiba et al., 2017). As a further perspective, it has been suggested that pre-mixing of samples and reagents and installation of a flow system would shorten the assay time and would enhance the performance of the proposed sensor as a suitable on-site tool (Ashiba et al., 2017). Most recently another approach for rapid and sensitive detection of norovirus was reported using an immunosensor based on long-period fibre gratings (LPFGs) with the detection sensitivity evaluated to be 1

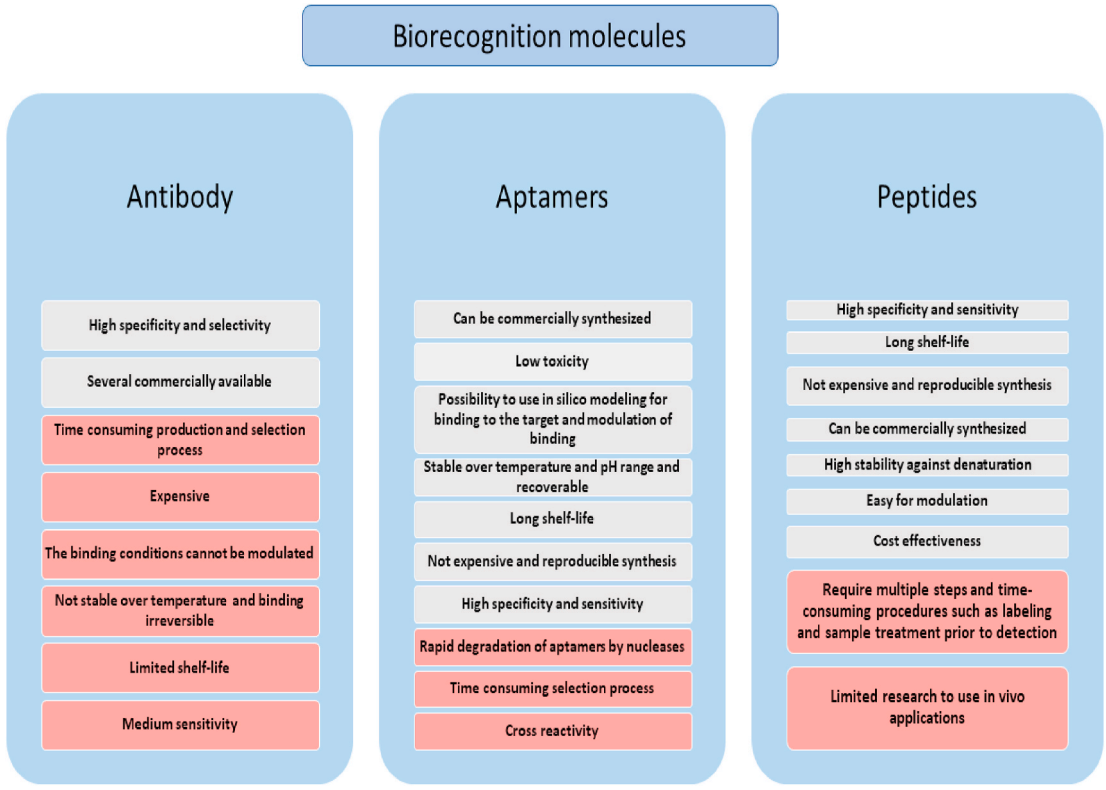

Fig. 3. Advantages (grey bold) and disadvantages (red bold) of biorecognition molecules used for norovirus biosensors including antibodies aptamers and peptides. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ng/mL of norovirus, and a total analysis time of 40 min in a label-free manner (Janczuk-Richter et al., 2020). Moreover, advanced nanotechnology approaches for ultra-sensitive detection of norovirus have been pointed out using a dual modality sensor using a liposome-based signal amplification technique (Ganganboina et al., 2020).

2.2.2. Aptamer-based biosensors

Aptamer-based biosensors (aptasensors), are one of the most sensitive and specific POCT methods for the detection of norovirus, and recently detection limits reaching low copies virus/reaction (~20 copies virus/reaction) and a high speed of reaction time of ~10 min have been reported (Weerathunge, 2019). Therefore, aptasensors are prospective methods for the development and detection of norovirus from food and clinical samples. In the study performed by Giamberardino and co-authors (the first group who reported the development and application of an aptasensor platform by proposing a lead DNA aptamer candidate) a selected aptamer (AG3) was developed against murine norovirus (MNV) using the Systematic Evolution of Ligands by EXponential enrichment (SELEX) screening method. After nine rounds of SELEX, AG3 posed a very high affinity binder, in the picomolar range, for MNV as well as for the synthesized capsid of human norovirus GII.3 (Giamberardino et al., 2013). AG3 posed a near million-fold higher affinity for norovirus detection in comparison to a non-specific DNA control sequence. Furthermore, the cross-reactivity tests confirmed a million-fold higher affinity for the noroviruses than for the feline calicivirus. To develop an aptasensor, AG3 was featured into a simple electrochemical sensor using a gold nanoparticle-modified screen--printed carbon electrode (GNPs-SPCE) that proposed a detection limit of approximately 180 virus copies/mL suggesting its possible on-site applications for the rapid detection of noroviruses in environmental and clinical samples. However, the authors suggested that optimization in a variety of matrices was required to ensure the effectiveness (Giamberardino et al., 2013). In a further study, another DNA aptamer with the affinity to bind to the norovirus GII.4 capsid protein VP1 was developed, however, it was not incorporated into a biosensing platform (Beier et al., 2014). Following this, Nakatsuka and co-authors reported a novel DNA structure, a split G-quadruplex (Gq) based DNA-nano tweezers (NT) was developed for norovirus detection as a model of signal generation with potential application in a portable sensor (Nakatsuka et al., 2015). A partial norovirus mRNA from GII-4 capsid was artificially synthesized and applied as a target molecule and the corresponding peroxidase activity measured by colorimetric response. A Gq molecule is a DNAzyme containing hemin as a co-factor required for its peroxidase activity and a guanine-rich sequence (Travascio et al., 1999, 2001) which therefore acts as a signal generator desirable for specific nucleotide detection (Roembke et al., 2013). This DNA-nano tweezers (NT) structure allows self-assemble from three single-stranded DNAs through simple mixing, and detects its target without requiring any washing steps. The target recognition sites of split Gq-based DNA-NT can be modified easily without the need to optimize the tweezers structure. This method could detect norovirus at the level of 4 nM in a homogenous assay without any competition or requirement of washing steps. However, the high background generated by the accumulation of free hemin was attributed for this limited sensitivity. It has been suggested by the authors that applying electrochemical detection and immobilization of split Gq-based DNA-NT onto an electrode will reduce the interference between peroxidase activity and hemin and therefore could lead to the improved sensitivity (Nakatsuka et al., 2015). Following this, a handheld MEMS-based electrochemical aptasensor device to target MNV was reported as a POC proof-of-concept solution using a previously selected aptamer AG3 (Giamberardino et al., 2013; Kitajima et al., 2016). In this study, an immobilized thiolated DNA aptamer on an on-chip gold (Au) working electrode was tested and the affinity between thiol and Au, and formation of a DNA aptamer monolayer was confirmed. However, the reported study did not indicate the limit of detection of this assay, as the sensitivity of this assay has not been optimized (Kitajima et al., 2016). Kim and co-authors reported a new rapid assay ('non-stop aptasensor'), guanine chemiluminescence-based on the principle of intra chemiluminescent resonance transfer (Intra-CRET) targeting norovirus GII. from water and artificial urine samples with the sensitivity evaluated to be 80 ng/mL of norovirus DNA (Kim et al., 2018). The highlighted simplicity and sensitivity of this assay relies on the application of a five guanine (5G) linker spacer combined with a DNA aptamer to capture norovirus GII. capsid. In contrast to other assays, the use of this aptasensor was time saving and it enabled a user-friendly approach, as there is no need for sample pre-treatment, long and multiple incubations and washings (Kim et al., 2018)]. Thus far, a colorimetric NanoZyme aptasensor is the most sensitive and rapid aptasensor reported to detect 20 copies of virus per reaction of murine norovirus (MNV) in 10 min (Weerathunge, 2019). This ultrasensitive, enzyme-mimic based catalytic activity of gold nanoparticles, colorimetric (inducing blue colour for positive reaction) aptasensor with high target specificity of an MNV aptamer, is comparable to the sensitivity of RT-qPCR targeting the infective virus dose of ID_{50} (18–1050 virus copies/mL). Therefore, such a device could replace the time consuming and lengthy 'gold standard' method (Weerathunge, 2019) Though it should be stated when leaving the sample preparation out of the equation, RT-qPCR can be completed in 60–90 min and there are now portable real time PCR platforms. It is the required sample preparation that prevents this approach being utilised in the field. Sample preparation, especially for food and clinical analysis, is a major consideration to the suitability of any methodology for POCT. New innovations in sample preparation methods or extraction methods for the virus being determined are in high demand. Additionally, the rigorous validation of any new methodology or approach should then be conducted using a blind panel of many different norovirus genotypes to determine the suitability of the test for its purpose.

2.2.3. Peptide-based biosensors

In comparison to previously reported antibody and aptamer-based biosensors, more recently designed peptide-based biosensors stand out for their sensitivity in the detection of norovirus. Hwang and others used a series of synthetic affinity peptides identified with evolutionary phage display applied on an electrochemical platform for GII.4 norovirus detection (Hwang et al., 2017). Among them, a specific short peptide called Noro-1 peptide showed high affinity for recombinant noroviral capsid proteins (rP2). The peptide then immobilized on a gold-surface and sensing was measured by three methods including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and quartz crystal microbalance (QCM). In CV and EIS, the impedance was obtained by varying the applied potential, however in QCM the frequency (regarding to mass and thickness) and dissipation (regarding to rigidity) were two factors measured simultaneously in real time (Wu et al., 2011). The biosensor selectivity was tested against rotavirus and proven to be highly specific for norovirus detection (Hwang et al., 2017). Using EIS, the limit of detection of Noro-1 biosensor was determined as 99.8 nM against rP2 protein and 7.8 copies/mL for norovirus in stool samples which was claimed by the authors to be the most sensitive assay reported for norovirus detection at that time (Hwang et al., 2017). More recently, an affinity peptide-guided based plasmonic biosensor has been proven as an efficient tool for the detection of norovirus (Heo et al., 2019). The high sensitivity of this assay was reported to be 9.9 copies virus/mL of norovirus capsid protein, however higher than that reported previously by (Heo et al., 2019). Thus far, the most sensitive biosensor reported is an electrochemical NoroBP-nonFoul (FlexL) 2-coated gold electrode sensor (Baek et al., 2019). This biosensor consists of the assembly of eight peptides separately on the gold electrode, which showed the highest binding affinity to norovirus by NoroBP peptide; due to the application of NoroBP peptide this is an ultrasensitive tool for detection of norovirus from food samples, with a limit of detection of 1.7 copies virus/mL (Baek et al., 2019).

2.3. Microarrays

Among the other POCT methods, microarrays (called as gene chips, biochips and DNA chips) have the great multiplexing potential to provide rapid genotype information which is valuable for monitoring not only the spread of norovirus but also several other GI viruses causing outbreaks as well their variations in circulating strains (Martinez et al., 2015). DNA microarrays involving thousands of micro reaction zones containing immobilized oligonucleotide sequences are implanted onto solid supports made from glass, plastic, or silicon which allows the simultaneous analysis of thousands of genes in a single experiment. This is the main advantage of microarrays over biosensors which only allow single measurements (Wang, 2000; Shoemaker & Linsley, 2002; Hahn et al., 2005).

In a study conducted by Jaaskelainen et al. (Jaaskelainen et al., 2006) a microarray method was applied for the simultaneous identification of genogroups and types of noroviruses and Astroviruses in stool samples. Pre-amplified monoplex- and multiplex-RT-PCR products were transcribed to single-stranded RNA (ssRNA) and hybridized to short detection primers. The fluorescent nucleotides were added to the hybridized RNA template by Reverse transcriptase enzyme and the fluorescence signal was measured by a microarray scanner. 13 norovirus GI and GII genotypes were assayed in stool samples whereby 45 samples tested positive for norovirus by microarray in contrast to 35 by a conventional PCR-method among a panel of 74 samples. Although the microarray detected all genogroups except one, the limitation of the microarray to detect emerging new types of norovirus and astroviruses should be considered in the primer design (Jaaskelainen et al., 2006). In another study by Pagotto and others (Pagotto, Corneau, Mattison, & Bidawid, 2008a, 2008b), a microarray based method was used for detection and analysis of the norovirus genome. A 917-bp RT-PCR amplicon spanning regions A-C (i.e. Partial polymerase and partial capsid) from GI.1 and GII.4 norovirus was applied. Sequences hybridized to an oligonucleotide array called NoroChip v2.0 which was developed for the simultaneous detection and molecular characterization of Norovirus (Pagotto et al., 2008a, 2008b). Brinkman & Fout, 2009 reported the development of a generic microarray for genotyping noroviruses by probe hybridization in water samples. In this tag array, RT-PCR amplicons were applied in a single base extension (SBE) reaction where genotype-specific probes were labelled and then hybridized to an Affymetrix GeneChip® GenFlexTM Tag Array for detection. The Single-Base Extension (SBE) reaction adds a single biotinylated dideoxynucleoside triphosphate (ddNTP) to the 3′end of a DNA duplex with a 5′ overhang resembling a PCR reaction (Fan et al., 2000). GenFlex tag-probes are bi-functional probes for labelling which can show the presence of a specific amplicon or hybridization of labelled probes in the microarray. In total, a variety of tag arrays were designed and their specificity was validated against 10 strains representing eight different GI and GII genogroups. The results of this initial validation were promising as each genogroup yielded a unique hybridization pattern. However, more comprehensive probe sets (covering all known genotypes) would be necessary for application in real samples (Brinkman & Fout, 2009, Robilotti, Deresinski, & Pinsky, 2015).

In a further study reported by Mattison and others, a NoroChip v3.0 was produced with oligonucleotides from 29 reference strains which hybridized to amplicons from regions B and C, as well as a 2.4 kb amplicon spanning regions A–D. Applying the longer region of AD amplicon provided better analysis of norovirus strains. Although, despite the successful validation, acquiring long, specific amplicons of all circulating norovirus strains poses a limiting factor for further application of NoroChip v3.0 as a standard strain typing method (Mattison et al., 2011). Chen et al. (2011) applied a first tiling microarray to detect and identify the genotype and strain of common food-borne viruses including norovirus in a single experiment. The tiling array against the commonly used re-sequencing microarray using four different oligonucleotides containing each base at the central position, requires one eighth the number of oligonucleotides by using overlapping oligonucleotides at two base intervals which make it a simpler array to identify emerging strains. The method developed without using PCR and oligonucleotides (probes) of each strain of viruses with 25 oligonucleotides long, were synthesized directly on the FDA-EVIR microarray by photolithography to have a total number of 91542 probes. Among that 18736 and 11468 probes used for norovirus genogroup II and I, respectively. The sensitivity of the array was estimated as 10,000 viral genomes (Chen et al., 2011). In another study by Thissen and others (Thissen et al., 2014) a multiplex version of the Lawrence Livermore Microbial Detection Array (LLMDA) was evaluated for the sensitive and specific detection of viruses in human clinical samples. The array identified rotavirus, norovirus and several Enterobacteria (Thissen et al., 2014). In a complementary study by Yu and others (Yu et al., 2016) a custom designed tiling microarray was tested for low-input food-borne viruses including human hepatitis A virus, norovirus and coxsackievirus without nucleic acid amplification. Therefore, a protocol developed by applying carrier cDNA to facilitate the effect of DNase I treatment used for fragmentation indicated the importance of proper usage of this enzyme especially in very low quantities of low-input viruses. The total number of probes on the array was 105,527 including control probes representing 18S and 25S ribosomal RNA. The sensitivity of the microarray for norovirus determined as 1.6×10^6 copies virus/reaction averaging with overall detection limit of 250–500 virus particles and could detect virus at subgenotype levels (Yu et al., 2016).

2.4. Immunoassays

TaqMan-based real time RT-PCR assays (i.e. RT-qPCR) are currently (i.e RT-PCR), are currently the most sensitive diagnostic tests approved for official control to detect norovirus from food and clinical samples (ISO 2019). However, several immunological assays, such as enzyme immunoassays (EIAs) and lateral-flow assays (LFA) have several benefits over PCR methods such as simplicity and cost-effectiveness. Furthermore, antibodies are among affinity ligands that can be developed specifically towards viruses for POCT methods and it has been proven that immunological tests could be more clinically specific than RT-PCR. (i.e RT-PCR detects asymptomatic norovirus shedding 28 days post-infection, whereas antigen testing with immunological methods will only detect norovirus 7 days post-infection) (Atmar et al., 2008).

2.4.1. EIA

In the need to develop effective methods, immunological methods including EIAs assays have been developed (Vinjé, 2015). Examples of those commercially available are presented in Table 2. Although these methods are high throughput and provide great specificity, the sensitivity level is low and time of analysis relatively long (Table 2). Moreover, the sensitivity and specificity of norovirus EIAs depends on the purpose and design of the test variability between outbreak or sporadic cases (Gray et al., 2007), which limited their usefulness for routine screening of samples (Vinjé, 2015). The commercially available kits such as IDEIA Norovirus EIA (Oxoid, Hampshire, United Kingdom), SRSV (II)-AD (Denka Seiken Co. Ltd., Tokyo, Japan), and RIDASCREEN (r-Biopharm AG, Darmstadt, Germany) provide the sensitivity of 65–80% while the specificity is around 90% which depends on the number of collected outbreak samples and the collecting time (Table 2). Therefore, because of the low sensitivity, interpreting test results from sporadic cases should be done with pre-caution (Costantini et al., 2010). Moreover, the negative samples from outbreaks need to be further confirmed by RT-PCR. It might happen when the viral titer is below the detection level as the limit of detection is above 10^6 viral particles per each gram of feces or there is the possibility that recognizing antibodies are less active toward specific norovirus strains (Costantini et al., 2010).

2.4.2. LFA

LFAs are membrane-based immunochromatographic tests designed

Table 2
Examples of commercially available norovirus LFA and EIA detection kits.

Method	Company	Country	Product	Limit of detection/Sensitivity [%]	Assay time [min]
LFA	R-Biopharm AG	Germany	RidaQuick Norovirus	92	15
	Nal van minden	Germany	Nadal Norovirus I + II	99	10
	Medix Biochemica	Finland	Actim Noro	99	20
	Meridian Healthcare	Italy	Immunoquick Norovirus	10^4 copies	15
	CerTest Biotec	Spain	CerTest Norovirus	12.5 ng/mL	10
	Operon, S.A.	Spain	Simple Norovirus	97	15
	Standard Diagnostics, Inc.	Republic of Korea	SD Bioline Norovirus	96.1	15
	Denka Seiken	Japan	QuickNavi™-Norovirus2	98.3	15
	Mizuho Medy Co., Ltd.	Japan	Quick Chaser-Noro	6.25×10^6 copies	5–10
	Eiken Chemical Co., Ltd.	Japan	Immunocatch Norovirus	N/A	15
	Nissui Pharmaceutical Co., Ltd.	Japan	GE test Noro Nissui,	N/A	5–10
	DS Pharma Biomedical Co., Ltd.	Japan	Rapid SP Noro	6.46×10^6 copies	5–10
EIA	Oxoid	UK	IDEIA Norovirus	3.1×10^6 copies	120
	Denka Seiken Co. Ltd.	Japan	SRSV (II)-AD	76.3	120
	r-Biopharm AG	Germany	RIDASCREEN	65	105

to confirm the presence or absence of a target analyte in a sample. LFAs are inexpensive, rapid (~15 min), and they do not require specialized equipment. They are comprised of a series of overlapping membranes and an immobilized conjugate; the conjugate is typically a recognition molecule (i.e. antibody) that has been conjugated to a visual marker (i.e. colloidal gold). The conjugate is very specific to the analyte of interest. Liquid sample is placed at one end of the assay, and capillary action pulls the sample across the length of the test. First the sample interacts with the conjugate marker to get 'tagged', and then it encounters one or more test lines. The tagged analyte then interacts with the test line to produce a result. A list of examples of commercially available LFAs for norovirus detection is presented in Table 2 identifying the supplier, detection limits and assay time for the analysis (Théry et al., 2016; Ushijima et al., 2017). R-Biopharm AG (Darmstadt, Germany) produces the RIDA®-QUICK Norovirus LFA for determining GI and GII norovirus in stool samples. R-Biopharm AG reports a 92% sensitivity and 98% specificity of the assay and available as a single test or multiplexed for simultaneous norovirus, rotavirus and adenovirus detection. Kirby et al. have independently evaluated the assay with 726 specimens and compared their results with RT-PCR used as the reference method (Kirby et al., 2010). In their study, Kirby, et al. (2010) reported a lower sensitivity (69%) and the same specificity. Similarly, in independent evaluations, Battaglioli, et al. (2012) reported a low sensitivity (61.4%), which they attribute to poor results with genotype 1 specimens. Ambert-Balay & Pothier, 2013 evaluated the assay with separate GI and GII samples and came to the same conclusion: the sensitivity for genogroup 1 noroviruses was only 17%, versus 64% for genogroup type 2 samples. Abbot (Princeton, New Jersey) produces an alternative, the SD BIOLINE Norovirus rapid test; this test also detects the presence of genogroup 1 and genogroup 2 noroviruses in faecal samples. Abbot reports 84.1% sensitivity and 96.1% specificity and independent evaluations are in agreement (Kim et al., 2012; Park et al., 2012). Furthermore, this assay can detect norovirus genotype 3 (81.8%) and genotype 4 (75.7%), although it is not advertised for this use (Park et al., 2012). That said, this assay also has a low sensitivity (23%) for GI norovirus (Ambert-Balay & Pothier, 2013). Denka Seiken (Niigata, Japan) produces an LFA with neonatal samples in mind. They first produced the QuickNavi™-Norovirus test, however, this had a high false-positive rate for samples and a low specificity for patients <4 months old (Takahashi et al, 2010, 2015; Tanaka et al., 2009; Thongprachum et al., 2012). The second iteration, QuickNavi™-Norovirus2 test kit was uniquely designed for use with neonatal stool samples and uses a swab that allows collection of samples directly from the rectum (Saito et al., 2014). The sensitivity and specificity of the QuickNavi™-Norovirus 2 test was calculated with from 172 samples compared to RT-PCR; the sensitivity is reported at 92% and the specificity 98.3%. Independent studies are in agreement with these, and further show that specificity is maintained irrespective of patient age (i.e. 0–12 months) (Takahashi et al., 2015). Hagström et al. improved the LFA limit of detection of norovirus by utilising phage nanoparticle reporters (Hagström, 2015). This assay improves the limit of detection 100-fold compared to conventional gold-labelled LFAs because the phage increases the binding area for labelled reporter antibodies. That said, this assay has only been trialled with non-infectious virus-like particles from genogroup 1 and more extensive validation needs to be done to verify sensitivity and specificity, especially in clinical samples. More recently, Doerflinger et al. developed a nanobody-based LFA that can detect virions from genotype 2 noroviruses (Doerflinger et al., 2016). This test maintains an equivalent sensitivity and specificity compared to commercially available tests (80% and 86% respectively), whilst reducing the assay time to 5 min. Uniquely, this assay could also detect non-infectious virus-like particles from other antigenically distinct norovirus clusters, including newly emerging strains. Most commercially available LFA tests have a reported limit of detection around ~10^6–10^8 copies/mL or ~5–6.5 × 10^8 copies/g stool (Ushijima et al., 2017).

2.5. Prospective methods

Several prospective POCT methods have been highlighted most recently for sensitive and specific detection of noroviruses. A promising POCT assay reported thus far, is a paper-based colorimetric cell-free system with synthetic antibody (synbody) based on viral enrichment for the detection of norovirus GII.4 Sydney from stool samples (Ma et al., 2018). In this POC assay, a combination of isothermal amplification and cell-free RNA sensing enable detection of 270 aM virus in a reaction. The advantages of this assay are its simplicity to differentiate positive and negative reactions through visual assessment of the sample while applying a simple method for concentration of virus particles using magnetic beads and norovirus DNA-binding synbodies with an increase in sensitivity with detection limits of 270 Zm (Ma et al., 2018). Innovative fluorescent microspheres combined on a test strip for two genogroups of norovirus (GI and GII.) are another interesting approach reported by Zhang and co-authors for robust and sensitive detection of the virus (Zhang et al., 2018). Such a fluorescent particles strip assay was designed using chromatographic materials and antibodies specific to norovirus (GI and GII) as a conventional double antibody sandwich assay with the sensitivity reported to be 4–8 times greater than qPCR (Zhang et al., 2018).

Additional state-of-art sensing/diagnostic tools and platforms for norovirus detection that have been discussed and have great potential for enhancing the ability of POCT include the microfluidics "lab-on-a-chip" devices (Chung et al., 2015), electrochemical based sensing platforms (Baek et al., 2019; Kitajima et al., 2016), molecularly-imprinted polymers (Sykora et al., 2015) and surface-enhanced Raman spectroscopy platforms (Achadu et al., 2020). A microfluidic chip module integrating different steps (i.e. cell concentration, cell lysis and RNA

extraction, NASBA amplification and detection) has been designed for the detection of Norovirus in oysters (Chung et al., 2015). An impedance electrochemical biosensor was developed using newly designed peptides as a rapid and sensitive platform for the discriminative detection of norovirus with a detection limit up to 2.47 copies/mL within only 30 min from real oyster (Baek et al., 2019). Sykora et al. (2015) used a virus-like particle (VLP) of norovirus from the predominant GII.4, and demonstrated that a virus recognition nanomaterial could be prepared by using VLPs as a safe substitute for the imprinting of a human pathogenic norovirus. These polymers integrated within different sensing devices can then be used a capture agents for norovirus detection or as sample preparation devices to remove different matrices. A novel biosensing system based on graphene-mediated surface-enhanced Raman scattering using plasmonic/magnetic molybdenum trioxide nanocubes was designed to detect norovirus via a dual SERS nanotag/substrate platform with a signal amplification of up to 10-fold with a broad linear range from 10 fg/mL to 100 ng/mL and a limit of detection (LOD) of similar to 5.2 fg/mL (Achadu et al., 2020). Although not currently described as a POCT the FilmArray multiplex enteric panel from Biofire is worth noting for the detection of 22 of the most common pathogens associated with GI including norovirus GI/GII. The easy-to-use, multiplex PCR panel system is a very sensitive test involving nucleic acid extraction and molecular detection all in 1 pouch without any risk of cross-contamination and the test can be completed in 1 h and for portability to point of site can potentially be run on a battery-operated device. Miniaturization of such a system for portability in the field could be a way forward.

Smartphones increasing offer advanced high tech miniaturised imaging and computing facilities. In study reported by Chung et al. (2019), a novel approach using a smartphone-based paper microfluidic particulometry was reported as an ultrasensitive method for detection of norovirus (1 copies virus/mL). Ultra-sensitivity of this method relies on a developed smartphone-based fluorescence microscope and an image-processing algorithm that identifies the particles aggregated by antibody−antigen binding, leading to an extremely low limit of norovirus detection (Chung et al., 2019). Already developed smartphone-based assays showing this approach have been reported as a prospective on-site tool that is available for fast and specific detection (Ding et al., 2019).

3. Conclusions

The development of POCT devices for the detection of norovirus contamination and outbreaks has been challenging due to the difficulties in culturing the human strain of norovirus in a cell culture dish and due to limitations in animal models. This not only hampered the development of diagnostic devices but has also inhibited further research on mechanisms of action and the development of therapeutics or vaccines. Compared to RT-PCR, several POCT methods such as isothermal amplification or EIAs can be rapid, less expensive and considered as a suitable tool in norovirus outbreak detection. However, commercially available EIAs for norovirus detection in stool samples don't provide enough specificity and sensitivity and require further confirmation by RT-PCR methods for negative samples. In contrast, isothermal amplification assays may have potential for the development of easy to use portable devices. A LAMP assay was able to detect norovirus with high sensitivity and specificity, but the challenge to use this assay relies on the applicability as a one-tube test with amplification performed in ambient temperature. Furthermore, none of the LAMP assays have yet been developed as commercially available kits for testing for norovirus. The novel innovative approach already introduced by ONT that could be applied for the detection of norovirus is by using an ONT LamPORE assay and MinION sequencing (ONT 2020). The assay was created for the fast and accurate detection of coronavirus and is a scalable and rapid method that is able to analyse approx. 15,000 tests per day with processing of 1–96 samples per hour and 1–768 samples per 3 h. This assay is combined with isothermal-amplification of LAMP primers, which is one of the most sensitive methods of isothermal amplification (ONT 2020). LAMP has been used successfully alongside the MinION for the analysis of a malaria parasite Plasmodium, leishmaniasis and dengue virus, providing a simple and fast way to amplify a specific target (ONT 2020). Thus far, another promising method of detection reported was combining isothermal amplification assays with other enrichment methods to detect norovirus. For example, NASBA and RT-RPA assays were successfully adapted on paper-based colorimetric cell-free systems combined with a synbody for enrichment and the sensitive detection of norovirus (Ma et al., 2018). Recently, biosensors (peptide-based and aptamers-based) were shown as competent alternative assays adapted on the portable devices of immunoassays. Isothermal amplification and RT-PCR, generate very rapid results, saving hours of sample analysis time and can be developed inexpensively into sensitive, specific tools that are easy to operate. Biosensors may be seen as a future gold standard for virus detection as they potentially can be developed inexpensively into simple, sensitive and specific analytical tools with the ability of very rapid analysis of multiple samples compared to the conventional methods (Altintas & Tothill, 2013; Cheng et al., 2013). However, more often there are obstacles with recognition molecules such as steric hindrance, denaturation and non-specific binding to nontarget molecules. Therefore, successful embodiment of the recognition molecule is critical (Caygill et al., 2010). Besides that, other obstacles such as samples preparation, system integration and sample matrix interference are still obstacles for biosensor technology (Sin et al., 2014).Therefore, as yet, none of these devices have been commercialized and *in vivo* tests using promising devices i.e peptide-based biosensors are limited to lab tests. Additionally, the diversity in the norovirus genotype and the antigenic drift of certain strains (e.g., GII.4) over time poses limitations in single binding event measurements for biosensor development for norovirus detection. Therefore, microarrays (high throughput biosensor substitutes) have greater potential for multiplexing, which can target several strains and could be considered as a powerful tool for detection and analysis of norovirus outbreaks. The limitations of these as POCT methods are currently their high operation costs and lower specificity/sensitivity due to the possibilities of non-specific binding or hybridization that may occur during amplification (Gilbride, 2014). To date microarray applications are limited to certain laboratories (Cheng et al., 2013) but there is increasing awareness and interest in these arrays or panels for multiplex analysis.

To conclude, regarding the advantages and disadvantages of the reviewed methods of detection, none of the current assays are flawless for norovirus detection. The already commercially available RT-qPCR methods are still highly comparable when making a choice of which method to use. However, a mass produced biosensor design, advances in biorecognition molecules in tandem with nanotechnology and combining microfluidics with smartphone technology, all provide advantages such as multiplexing, reducing sample size and ease of fluid control and process data automation for futuristic diagnostic devices.

Among the handheld devices microarrays have the greatest potential for the multiplex detection and genotyping of norovirus. Microarrays appear to be the preferable platform for development to screen multiple samples in the case of outbreaks or for food security. However the cost and equipment challenges should be overcome. Though the importance of full validation of the POCT to be fit for purpose should never be forgotten and should be vigorously tested at POC to accredited standards.

This review sought to examine and evaluate the most suitable POCT methods for the efficient detection of noroviruses and highlighted the advantages and disadvantages of the reported methods in the peer-reviewed literature. However, the need for the development of SMART technologies for the robust detection of other important viruses causing disease outbreaks in plants, animals and humans, could make the technological applications presented here model systems for other viruses to assure human health and food safety.

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