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Research Progress on Rapid Detection Methods Based on Multiple Norwalk Viruseses

Wenying Zheng, Jiatong Lai, Junjie Liu, Liyan Wu*

Zhuhai College of Science and Technology, School of Pharmacy Food Science, Zhuhai, Guangdong, 519041, China
**corresponding author*

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Abstract: Norwalk Viruses (NV) is a calciferous causing acute gastroenteritis, which is generally susceptible, low in infection dose, short in latent time and highly contagious, and has large and small outbreaks all over the world every year. Studying and comparing the existing detection methods and finding the fastest and most efficient detection method in different clinical situations is of great significance for laboratory virus detection and hospital clinical diagnosis. In this paper, several common detection techniques of Norwalk Viruses were reviewed, and their advantages and disadvantages were compared, providing feasible ideas and reference for laboratory research and clinical diagnosis and treatment.

1. Introduction

For example, Norwalk Viruses, an infectious disease spreading around the world, is a cup-shaped virus, which causes acute gastroenteritis in infected people by contaminating food and water. It is generally susceptible to non-special people, with low infection dose, short incubation time, usually less than 48 hours, and strong inactivity. Because of its rapid variation and complicated infection pattern, it is easy to be mistaken for bacterial acute gastroenteritis and cause virus outbreaks. Therefore, we are seeking to find suitable detection technology, which is extremely important to prevent the spread of diseases caused by Norwalk Viruses. In this paper, several conventional methods for the detection of Norwalk Viruses are reviewed, including gene chip detection based on PCR amplification, bio-sensor technology and cinematography technology. In addition, the collection and transportation of high-quality samples play an important role in the detection results. Experiments have proved that the best sampling material for virus detection is the feces of infected individuals, followed by vomit [1].

2. Chemical Structure and Transmission Routes of Norwalk Viruses

Norwalk Viruseses are non enveloped, single stranded, positive strand linear RNA's, and Norwalk Viruseses are tetrahedral symmetric spheres with no capsule. (figure 1) its genome encodes three open reading frames, ORF 1, ORF 2, and ORF 3 sequentially from the 5 'end to the 3 'end. Among them, ORF 2 and ORF 3 encode the major capsized protein VP 1 and the minor capsized protein VP 2, respectively, which are the main protein molecules constituting virus particles. Six protein

molecules (p 48, NTP, p 22, VPG, pro, pol γ) after ORF translation play a role in virus genome replication and transcription. According to the difference of Norwalk Viruses protein coding sequence, it can be divided into six categories: Gi, Gii, Giii, Giv, G VV and G VI, among which Gi and Gii Norwalk Viruses cases are the main groups. Gi and Gii were further divided into 9 genotypes and 22 genotypes, respectively [2].

Figure 1. Norwalk Viruses structure

For example, Norwalk Viruses can be transmitted through food, water, aerosols and feces of vomiting patients, and the virus is constantly eliminated during infection process. The tolerance to the visual environment is also strong, which is easy to cause widespread infection. Common shellfish such as scallops, mollusks, and Philippine clams, as well as fruits and vegetables that have not been strictly treated, may all cause viral infection. After infection, the symptoms are acute gastroenteritis, with obvious seasonality, and the incidence rate is higher in cold seasons, also known as "winter vomiting".

3. Detect Norwalk Viruses with Electron Microscope

The detection of Norwalk Viruses by electron microscope (EM) is the most primitive method to detect Norwalk Viruses, among which EM detection can be divided into direct EM detection (EM) and immune detection (IEM) [3]. Electron microscopy is the use of labeled antibodies that bind to viral surface antigens, allowing visualization of the trapped content virus. The detection of Norwalk Viruses requires a high concentration of Norwalk Viruses carried by the sample, usually requiring the virus concentration of the sample to be higher than 105/cm /cm 3. Due to the relatively high requirements of equipment, and due to the sample concentration and requirements, such as the operator's technical experience and the conventional judgment of Norwalk Viruses, the specificity and sensitivity of EM detection of Norwalk Viruses are relatively low (Figure 2.).

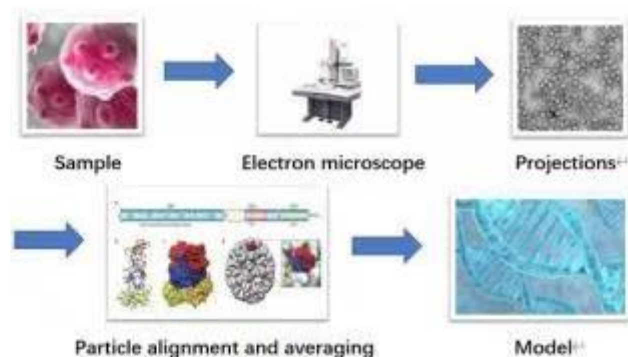

Figure 2. flow of electron microscopy assay

4. Detection of Norwalk Viruses by Reverse Transcription Polymerase Chain Reaction (Rt-Pcr)

The RT-PCR assay is one of the most common assays for Norwalk Viruseses [4]. In recent years, the case detection shows that conventional RT-PCR has solved the problems of laborious and high cost in the previous detection. At the same time, this technique has a high mutation rate in the detection of Norwalk Viruses, but its use has been popularized after the introduction of a variety of specific primers and probes.

4.1. Routine RT-PCR Test

After the RNA of the virus is reversed and the cDNA is synthesized (i.e. MRNA into cDNA), and then we carry out PCR amplification to realize gene expression, thus improving the sensitivity of detection. Through sequence comparison, the specificity of the test was improved. After using RT-PCR to detect Norwalk Viruses, the experimenter can immediately determine primers of RNA polymerase gene region. Due to the dependence of RNA on this primer, some experimenters would sample diarrhea people according to the conditions at that time, sequence the sample, and analyze the comparison in the DNA sequence database. By comparing the maps, they would find that Norwalk Viruses is positive. The appearance of this method laid the foundation for subsequent detection and diagnosis of Norwalk Viruses, and opened up another research way for Norwalk Viruses detection.

4.2. Multiple RT-PCR Detection

This method can detect different sub types of Norwalk Viruses, and its popularization and use can save time and detection cost. The biggest advantage is that it can detect multiple viruses at the same time, while the disadvantage is that its sensitivity is relatively low. The detection process is that different fragments of the same DNA template or different templates are amplified in the same reaction system, and different specific primers are needed in the detection process. In some experimental cases, the researchers tested stool samples of about 10 kinds of diarrhea-causing viruses according to the test requirements, and designed PCR primers, which led to different lengths of amplified product fragments, which led to different types of viruses.

Experimental test data of shigemoton and other researchers show that the detection rate of this method in Norwalk Viruses samples is as high as 96.7%. Subsequently, this method was widely used to detect Norwalk Viruses in many pathogens. The popularization of this method is of great significance to the detection and clinical diagnosis of Norwalk Viruses infectious diseases [5].

4.3. Fluorescence RT-PCR Detection

Fluorescent RT-PCR, that is, target probes are coupled with fluorescent dyes, is commonly used to detect different nucleic acid types, target probes are specific, and the method has high specificity and sensitivity. The amplification products can be generated at any time during the PCR cycle in the reflection phase. Targeted primers used to detect the genotype of Norwalk Viruses exists in Norwalk Viruses, but due to the position of the primer in the conservative region, the variability of single nucleotide of Norwalk Viruses and the inability to ensure the sensitivity and accuracy of the reaction, the probes and primers were adjusted appropriately.

For example, miurat and other researchers designed three pairs of different primers for several groups of feces [5]. During this period, they also added a synthetic fluorescent dye, which effectively improved the specificity and sensitivity of the reaction. Based on this technology, a variety of detection kits have been developed, and they have been used in clinical diagnosis and laboratory research on a large scale.

5. Microarray Detection of Norwalk Viruses

Globally, the main pathogens causing diarrhea are bacteria and viruses, among which rotavirus and calicivirus are the most common virus types. In recent years, gene chip technology has gradually been used to detect Norwalk Viruses.

According to current research progress, the DNA micro-array (gene chip) is an application of micro-array technology, which is mainly used for qualitative or quantitative measurement of nucleic acids existing in living organisms. The probes used for qualitative and quantitative purposes are composed of several KB (cDNA chip) or dozens of specific types of nucleotides. An important principle underlying the viability of this technique is the complementary pairing interaction between bases and the hybridization between single strands of complementary nucleotides. According to the hybridization system, it can be divided into solid-phase gene chip technology and liquid-phase gene chip technology [6].

5.1. Liquid Chip Technology

Norwalk Viruses detection based on liquid-phase chip technology: Liquid-phase gene chip technology, also called suspension gene chip technology or liquid-phase gene chip technology, makes use of the research and detection of Norwalk Viruses by Ya Qing et al. The researchers used multiplex RT-PCR combined with liquid chip technology to detect Norwalk Viruses in the samples. PCR products were hybridized with the mixture of nucleic acid probe microspheres coupled to these viruses, and these viruses were detected by liquid chip detector [4].

Now, Chung and other researchers have detected Norwalk Viruses in oysters by using liquid-phase gene chip technology and electrostatic adsorption technology. The liquid-phase gene chip detection method developed by Luminex Company of the United States is based on the principle of microsphere design. It can provide accurate and repeatable detection results for each index, only needs a few sample, is cheaper and successfully obtains more data than other detection methods in a short time. Luminex immunoglobulin isotype analysis can quantify up to six immunological subtypes at the same time. According to κ, λ chains, immunological sub types can be quantified in cell culture supernatants, serum or plasma samples, and their rapid and accurate imprinting function has been favored by many scientific research institutions.

5.2. Visualization of Magnetic Beads by Gene Chip Technology

The biggest advantage of this technology is that we can recognize it with the naked eyes, and the detection results are more intuitive and vivid. The principle is that biotin specifically binds to streptomycin. (There is a demimondaine ring in the molecular structure of biotin, which is the main binding site of streptomycin (A) or streptomycin (SA). The other tetrahydrothiophene ring has a side chain on c 2, and its terminal hydroxide group is the only structure that is bound with proteins. Biotin and streptomycin have been introduced in immunohistochemistry, ELISA and nucleic acid hybridization technology, and can be used for various items of detection. They use biotin to label sample nucleic acids, and streptomycin to encapsulate markers such as magnetic beads or Kano-metals. Different colors are produced by streptomycin affinity with biotin. Shi Lei et al.'s research. As an example, firstly, biotin-labeled gi and gii primers were used to detect and amplify the clinical trial samples. Under this operation, the PCR products of biotin-labeled gi and gii are combined with the complementary probes of gi and gii on the chip to form stable double strand. Then, the streptomycin-coated magnetic beads were hybridized, and the results were obtained by naked eyes or ordinary microscope.

The research and development of this technology has improved the shortcomings of high cost and high expenditure of florescent labeled chip technology, and can make many enterprises popularize the application of chip technology on a wider range.

6. Using Biosensor Technology to Detect Norwalk Viruses

6.1. QCM

Since this technique uses quartz crystals as sensors, also known as noncrystalline sensors, its working principle is that the mass of adsorbed species on the chip surface is converted into a frequency signal. Steps: First, we screen out binding pep-tides with good affinities to retrovirus proteins through related technical methods (transcription, reverse transcription, labeling, etc.), and then selected related substances (such as thole-modified gold particleboard) to form self-assembled mono layer binding according to laboratory or local economic conditions, so that we can detect Norwalk Viruses by QCM. The method has the advantages of high detection efficiency, low molecular weight and low detection limit.

6.2. Technology of Ion Resonance Sensor

Monitoring the dynamics of microorganisms in real time. Van der Waals forces, hydrogen bond formation, hydrophobic forces, torque forces, and so on will be closely related to the results of the assay, so we need to monitor and judge the kinetic status of microorganisms during the detection of Norwalk Viruseses, so the world began to apply this biochemical sensor to detect related viruses, in which the detection of Norwalk Viruseses was a major breakthrough just after the advent of this technology.

We can screen single-stranded nondeductible DNA that specifically binds to Norwalk Viruses and emits fluorescence. They are used as aptamers. After processing, aptamers would be adsorbed on the gold film surface of carbon electrode due to bonding force. When Norwalk Viruses flows through SPR, it would change the signal of SPR, and the specific binding of aspartame would produce the effect of detecting Norwalk Viruses. This method has low purity requirements for samples, has the characteristics of high specificity and sensitivity, small molecular weight of tamper, simple structure, being able to be used repeatedly, and long shelf life.

6.3. ATP - as

We can use energy conversion to detect Norwalk Viruses, which is a way to save energy and cost. The characteristic of 1 ATPase that can convert energy in living organisms points out a research direction for this idea. And f 1 can be driven by physiological processes (proton trans membrane and ATP hydrolysis) in living organisms. We can first design probes such as the e sub-unit antibody biotin streptomycin biotin Norwalk Viruses probe so that its binding to the sensor constitutes a bio-sensor, according to the principle that the probe will recognize Norwalk Viruses after it causes sensor activity and thus synthesize different ATP, and finally we only need a detection ATP reagent to detect the result.

Because the recognition of Norwalk Viruses is caused by the activity of 1 ATP as changed by the synthesis of different ATPS, finally, we can detect the results only by detecting ATP reagent. This method has the advantages of simple instruments, low cost, high working efficiency and no need to extract nucleic acids, so it has been widely used by research enterprises.

7. Immunochromatographic Methods Used to Check Norwalk Viruses

Immune colloidal gold technology is a new immunolabeling technology, which uses colloidal gold as a tracer of antigens and antibodies. Colloidal gold is a stable colloidal state formed by the polymerization of chloroauric acid (Haul 4) in the presence of reducing agents such as white phosphorus, ascorbic acid, sodium citrate, tannic acid, etc. Under electrostatic action, which is called colloidal gold. Gold immunochromatography (GICA) is a kind of solid-phase immunolabeling technology, and the most common application being colloidal gold immunochromatographic test paper. Colloidal immunochromatographic test strips are widely used in various fields, and have a series of advantages, such as simple operation, short detection time, and no need for professional operation, etc., which are especially suitable for clinical detection and large-scale epidemiological investigation [7]. At present, Norwalk Viruses test strips are popular.

In existing research, immunochromatographic verification is aimed at gii virus. First, dilute antiviral antibodies is prepared, and fluorescent reagents (usually dylight) is added as labels, and then immunochromatographic test strips with scraping film is used as T line. Chromatographic buffer can't be directly mixed with virus, so it should be diluted 50 times first, placed in a constant temperature incubator, mixed and placed, and scanned with infrared fluorescence instrument the next day. If the fluorescence peak appears in the area of T-line, the virus test is positive [8].

8. Summarization and Outlook

To sum up, the electron microscope method is a preliminary detection method, and the typing of Norwalk Viruses is determined by observing the virus morphology by electron microscope. But the required virus concentration is high, and the operation requirements are high. Because it can only be carried out in professional testing institutions, this technology can not be widely used.

Molecular biology (RT-PCR technology has high sensitivity and specificity in detecting Norwalk Viruses. Generally, the detection results of RT-PCR technology can be used as standard results of this sample, which is fluorescent and multiple. However, the molecular biological detection technology requires high skills of equipment and personnel, and base sequence comparison takes a long time, which is not conducive to rapid emergency response when large-scale cases break out.

The gene chip has high technical flux, and can realize the automatic detection of Norwalk Viruses typing and simultaneously detect various pathogens. However, there are still some problems, such as immature manufacturing technology, collection of probe sequences on the chip, etc., and there is much room for development in the future.

Biosensor technology can be reused to detect Norwalk Viruses with high efficiency and low pollution. However, at present, this technology is not mature and perfect, and it is unlikely to be applied on a large scale.

The immunochromatographic detection method has short time and is easy to operate. ELISA and colloidal gold immunoassay can be used as a rapid detection method for Norwalk Viruses detection for the first time, but the accuracy of immunochromatography detection method is not high, and its sensitivity and specificity need to be improved.

Immunochromatographic test results are easily influenced by sample contamination, so it is impossible to accurately type Norwalk Viruses. However, because the antigen detection is fast, simple, safe and sanitary, it doesn't need the assistance of instruments, and the detection price is cheap. Because Norwalk Viruses is easy to spread, mutates quickly, and its infection pattern is complex, it is easy to cause large-scale outbreaks in dense places. Therefore, it is necessary to develop more efficient and rapid detection methods in the future. At present, for Norwalk Viruses detection, all localities should make flexible use of local resources, establish a rapid Norwalk Viruses detection process, and form a complete Norwalk Viruses detection system, which will have a far-reaching impact on local medical systems.

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