Laboratory Diagnosis of Noroviruses: Present and Future

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KEYWORDS

• Norovirus • Genotypes • Sporadic and outbreak gastroenteritis • Laboratory test • Diagnosis

KEY POINTS

- There is no test for infectious norovirus (NoV) because of the absence of *in vitro* culture system.
- Electron microscopy is no longer an adequate detection method for NoV.
- The highly diversified genome and antigens of NoV present challenges for the development of antigen-based immunoassays.
- Real-time reverse transcription polymerase chain reaction (RT-PCR) is currently considered as the gold standard test for NoV.
- Rectal and oral swabs, which are convenient to collect, may be good alternatives to stool samples for testing of NoV.
- Genotyping is an important tool for NoV molecular epidemiologic surveillance and vaccine development.
- Further development of nanotechnology-based platforms might provide point-of-care testing.
- Each laboratory needs to select a testing platform that is best fitted for its needs and feasibilities.

INTRODUCTION

With the development and application of advanced diagnostic assays to detect norovirus (NoV) in public health and clinical laboratories, NoV is well known as one of the top 5 causative agents of global epidemics of gastroenteritis outbreaks and is increasingly being recognized as one of the most important pathogens of sporadic gastroenteritis.[1,2]

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Based on a systematic review of 31 studies in both developed and developing countries, it was estimated that NoV accounted for 10% to 15% of severe gastroenteritis cases in children younger than 5 years and 9% to 15% of mild and moderate diarrheal disorders in populations of various ages, leading to 1.7 million to 1.9 million outpatient visits and 19 million to 21 million total illnesses per year in the United States.[3] NoV is becoming the predominant cause of sporadic gastroenteritis in young children, especially in regions where rotavirus vaccine programs have been implemented.[4,5]

Laboratory testing for NoV has undergone development during the last 4 decades since the virus was discovered by Dr Kapikian using immunoelectron microscopy (EM).[6] There are still knowledge and technical gaps, with no method to detect infectious NoV particles because of the absence of *in vitro* viral culture system and small animal model. Although research laboratories can produce recombinant p particles and viruslike particles (VLPs) that have NoV characteristics and can be used as a vehicle for vaccine development,[7,8] the inability to produce large volume of naturally occurring viral particles hampers the development of diagnostic assays. At present, real-time RT-PCR is considered the best assay to detect NoV in both research and clinical settings. However, there are still limitations with this approach, including the need for multistep procedure, high cost, need for special instruments, and being too sensitive to provide results relevant to clinical situations. Simple, more affordable, and rapid testing methods such as enzyme immune assay (EIA) or enzyme-linked immunosorbent assay (ELISA) to detect NoV have been developed but generally have low sensitivity and thus limited utility. Novel nanotechnology array-based assays to detect NoV are in development and under validation. A major breakthrough with nanotechnology array-based assays will be the development of point-of-care tests, featuring rapidity and potential utility in many settings including resource-poor countries depending on affordability. It will take time to validate some of these prototypes before their application in clinical diagnostics. The future directions of technology development for NoV detection likely focus on method simplification, cost-effectiveness, analytical precision, and accuracy. This review summarizes technologies used in the detection of NoV and highlights some of their features.

NOROVIRUS AND ITS CLINICAL RELEVANCE

Structures and Taxonomy

NoV is a small (30–38 nm), round, nonenveloped virus with a single-stranded, positive-sense RNA genome. NoV was initially called small round-structured virus, deriving from the smooth surface of its morphologic structure.[9] Using cryoelectron microscopy and x-ray crystallography analysis of VLPs, the 3-icosahedral capsid of NoV was shown to be composed of 90 dimeric capsomers made up of 180 capsid proteins. Each capsid protein possesses 2 major domains, the shell and the protrusion domains. The RNA genome of NoV is ~7.5 kb in length with a poly A tail.[10] Three open reading frames (ORFs) have been characterized, with ORF1 encoding the nonstructural proteins, ORF2 the VP1 capsid protein, and ORF3 a minor structural protein.

NoV family is classified into 5 genogroups (GI-V) with GI, GII, and GIV found mainly in human infections, GII and GIII in pigs and cattle, and GV in mice.[11] Zoonotic transmission of NoV has been postulated only based on seroprevalence studies.[12] At least 14 genotypes of GI, 29 genotypes of GII, and 2 genotypes of GIV have been identified.[13–15] Most human infections are caused by NoV GI or GII. Up to 85% of global epidemics of NoV infections have been caused by different GII.4 (GII genotype 4) variants that emerge every 2 to 3 years. NoV GII.4 was found to have a higher mutation rate and