JCM
Journals.ASM.org

Advances in Laboratory Methods for Detection and Typing of Norovirus

Jan Vinjé

Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Human noroviruses are the leading cause of epidemic and sporadic gastroenteritis across all age groups. Although the disease is usually self-limiting, in the United States norovirus gastroenteritis causes an estimated 56,000 to 71,000 hospitalizations and 570 to 800 deaths each year. This minireview describes the latest data on laboratory methods (molecular, immunological) for norovirus detection, including real-time reverse transcription-quantitative PCR (RT-qPCR) and commercially available immunological assays as well as the latest FDA-cleared multi-gastrointestinal-pathogen platforms. In addition, an overview is provided on the latest nomenclature and molecular epidemiology of human noroviruses.

Norovirus is a good example of a pathogen where improved diagnostics has increased its recognition from a relatively unknown virus before the mid-1990s to the leading cause of epidemic and sporadic gastroenteritis in people of all ages worldwide (1, 2). The majority of norovirus outbreaks occur in health care settings (including long-term care facilities and hospitals), where the virus is predominantly spread from person to person. In addition, noroviruses have also been identified in over 58% of the reported foodborne outbreaks in which an etiologic agent was determined (3). In the most recent disease burden estimates in the United States (US), norovirus causes 570 to 800 deaths, 56,000 to 71,000 hospitalizations, 400,000 emergency room visits, and 1.7 to 1.9 million outpatient visits annually (4). In pediatric populations in industrialized countries where a rotavirus vaccine has been introduced, noroviruses are rapidly replacing rotavirus as the most common cause of medically attended acute gastroenteritis (2, 5). After an incubation period of 12 to 48 h, norovirus illness is characterized by projectile vomiting, nonbloody diarrhea, nausea, abdominal cramps, and low-grade fever. Some persons might experience only vomiting or diarrhea. In healthy individuals, the duration of symptoms is usually not longer than 48 h, and the disease is self-limiting in most patients. However, young children and the elderly are at increased risk for more-severe and prolonged illness leading to hospitalization, while the disease is increasingly recognized as an important cause of chronic gastroenteritis for immunocompromised patients (6). In countries that belong to temperate latitudes, most infections occur in the fall and winter and at least 70% of outbreaks are reported in semiclosed communities such as long-term-care facilities, schools, hospitals, and cruise ships. Noroviruses can infect humans via multiple routes, including the oral route, transmitted through contact with fecal matter or aerosolized vomitus from infected people, as well as contaminated surfaces, food, or water. Upon infection, noroviruses replicate in cells in the upper small intestinal tract (duodenum and upper jejunum), leading to both epithelial barrier and secretory pathway dysfunction. T cells are likely required for virus clearance from the intestine, and, as was reported in a case study of an immunocompromised patient, after more than 1 year of chronic norovirus diarrhea, increasing levels of T cells were associated with resolution of symptoms (7). Outside the human host, the virus is environmentally stable and has an estimated 50% human infectious dose (HID_{50}) ranging from 18 to 1,015 genome equivalents, although a recent study estimated that the HID_{50} is more similar to those of other RNA viruses (1,320 to 2,800 particles) (8). This article reviews methods based on antigen and molecular detection of human noroviruses. Although other molecular methods such as those employing isothermal amplification (nucleic acid sequence-based amplification [NASBA], loop-mediated isothermal amplification [LAMP]) and microarrays have been described, this review focuses on immunological and reverse transcription-PCR (RT-PCR)-based molecular methods.

Noroviruses are a group of nonenveloped single-stranded positive-sense RNA viruses classified in the family *Caliciviridae*. The virus particles are 27 to 40 nm in diameter, and the genome is 7.5 to 7.7 kb in length and, except for murine norovirus, contains 3 open reading frames (ORF1, ORF2, and ORF3). ORF1 encodes a polyprotein that is posttranslationally cleaved into seven nonstructural mature proteins (NS1 to NS7) that are involved in viral replication. ORF2 encodes the major structural protein (VP1) of approximately 60,000 Da, and ORF3 encodes a minor structural protein (VP2). The viral capsid contains 90 dimers of VP1 and a few copies of VP2. X-ray crystallographic structure studies using Norwalk virus-like particles have revealed that the VP1 has a shell (S) and a protruding (P) domain (9). The S domain surrounds the viral RNA, and the P domain, which is linked to the S domain through a flexible hinge, corresponds to the C-terminal part of the VP1. The P domain is further divided into P1 and the highly variable P2 subdomain which contains the putative neutralization sites and interacts with histoblood group antigens (HBGAs). VP2 is located interior to the virus particle and is most likely involved in capsid assembly and genome encapsidation (10).

Except for murine strains, noroviruses cannot be cultivated *in vitro*, which prevents their classification into distinct serotypes. Therefore, they are genetically classified into 6 established geno-

Accepted manuscript posted online 2 July 2014

Citation Vinjé J. 2015. Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol 53:373–381. doi:10.1128/JCM.01535-14.

Editor: G. V. Doern

Address correspondence to jvinje@cdc.gov.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.01535-14

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

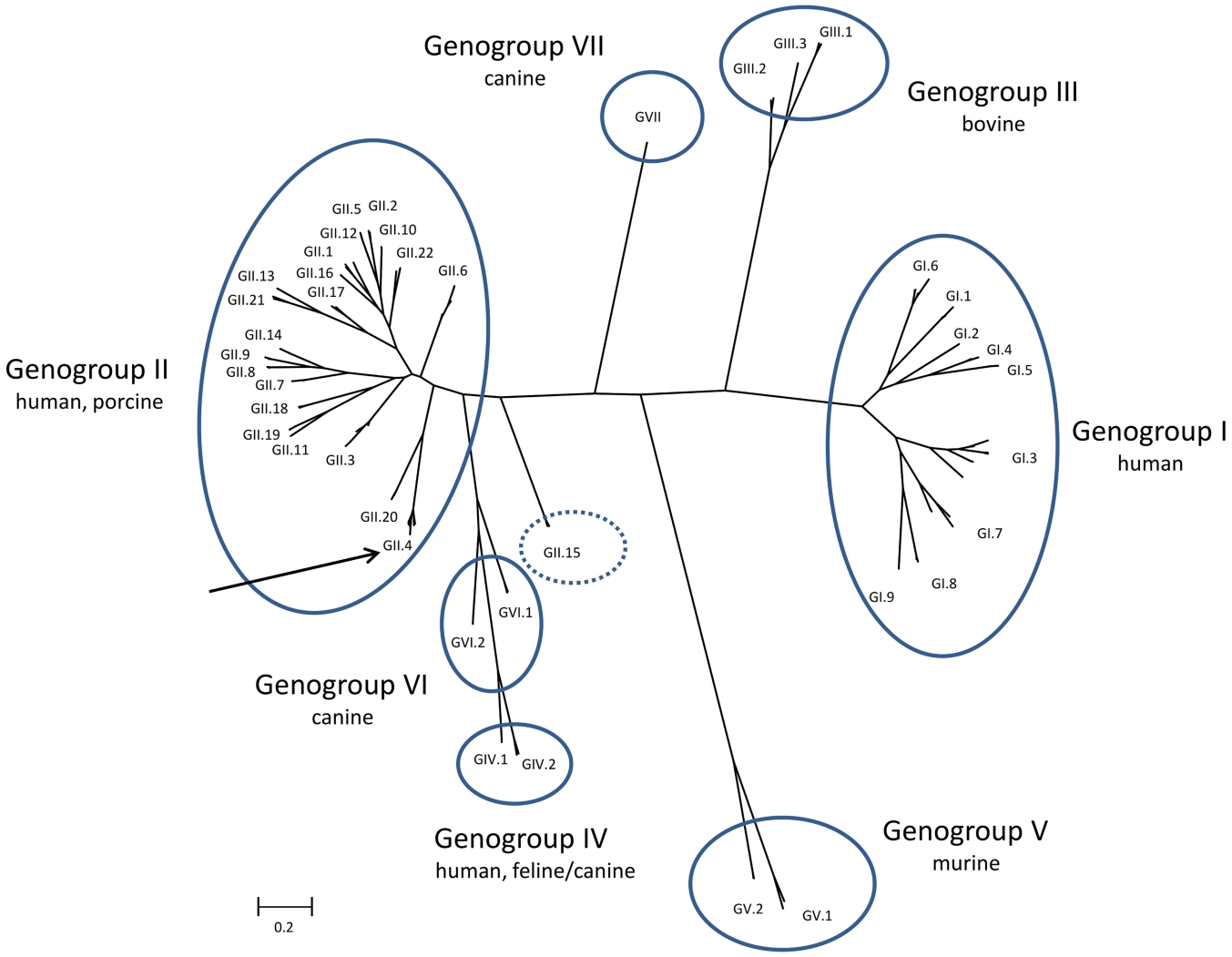


FIG 1 Classification of noroviruses into 7 genogroups (GI to GVII) based on amino acid sequence diversity in the complete VP1 capsid protein. To build the phylogenetic tree, capsid sequences from 105 strains representing the spatial and temporal sequence diversity of noroviruses from diverse geographic regions across the world were selected. Viruses belonging to GI, GII, and GIV infect humans, except GII.11, GII.18, and GII.19 viruses, which infect porcine species, and GIV.2 viruses, which infect canine species. GII.15 viruses, which have been detected only in humans, form a tentative new genogroup (dotted circle). GIII viruses infect cows and sheep, GIV.2 infects canines, GV.1 and GV.2 infect mice and rats, respectively, and GVI and GVII infect canine species. GII.4 viruses (arrow) are responsible for the majority of norovirus infections worldwide. The scale bar reflects the number of amino acid substitutions per site.

groups (GI to GVI) (11), while tentative genogroup VII is proposed in this paper (12) (Fig. 1). GI and GII viruses are responsible for the majority of disease in humans, whereas GIV viruses are rarely detected as the cause of epidemic or sporadic disease. Based on the most recent phylogenetic analysis, GII.15 viruses may need to be reclassified as a separate genogroup, but this would need consensus approval from the international norovirus working group (13). Each genogroup is based on phylogenetic clustering of the complete VP1 amino acid sequence and is further divided into genotypes (13, 14) (Fig. 1). To date, nine capsid genotypes have been recognized in GI and 22 in GII, and three genotypes of GII (GII.11, GII.18, and GII.19) have been uniquely detected in swine. GIV viruses consist of 2 genotypes, of which GIV.1 has been detected in humans and GIV.2 in feline and canine species (15). GII viruses are most frequently detected (89%), whereas GI viruses, which include virus of the GI.1 prototype Norwalk virus strain, cause approximately 11% of the outbreaks (16).

Despite the extensive genetic diversity among noroviruses, viruses from a single genotype, GII.4, are responsible for the majority of the norovirus outbreaks worldwide (17). Due to epochal evolution, novel pandemic GII.4 variants have emerged every 2 to 3 years since the mid-1990s, replacing previous predominant GII.4 strains but not other endemic strains (17). These global GII.4 variant strains include the GII.4 US95/96 strain in 1995, GII.4 Farmington Hills in 2002, GII.4 Hunter in 2004, GII.4 Den Haag in 2006, GII.4 New Orleans in 2009, and GII.4 Sydney in 2012. These new GII.4 variants are often, but not always, associated with an increase in the number of outbreaks (18). In the United States, GII.4 Sydney has continued to cause the majority of the norovirus outbreaks during the 2013-to-2014 season (unpublished data).

Several mechanisms that could explain the evolution of GII.4 viruses have been proposed, including the host herd immunity that drives antigenic variation in the hypervariable P2 domain of VP1. This domain of the viral capsid binds HBGAs, which serve as

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

TABLE 1 Overview laboratory assays for detection of norovirus

Laboratory test(s)	Advantage	Disadvantage	Time (sample to result)	FDA (510k)-cleared test	Market
Electron microscopy	Ability to detect multiple viral pathogens	Expensive equipment and training; low throughput; insensitive	15 min		Reference laboratories
Immunological					
Enzyme immunoassay	High specificity, high throughput	57–76% sensitivity	60–90 min	R-Biopharm	Public health, clinical laboratories
Immunochromatographic	High specificity, no special equipment Single sample can be tested	35–52% sensitivity	15 min		Point of care
Molecular					
Conventional RT-PCR	PCR amplicons can be sequenced and used for typing	Results must to be confirmed by sequencing or hybridization	5–6 h[a]		Reference laboratories
Real-time RT-PCR	High specificity, sensitivity and throughput; possibility to multiplex mulitple targets	PCR equipment required; reduced clinical specificity	3 h[a]	Tests in pipeline	Public health, clinical laboratories
Molecular multiple enteric pathogen					
xTAG GPP	High sensitivity, high throughput; detects 11 different enteric pathogens	Expensive equipment and kit format	5 h[a]	Luminex Corporation	Public health, clinical laboratories
FilmArray GI Panel, Verigene Enteric Pathogens Test	Includes nucleic acid extraction; detects 23 (FilmArray) and 9 (Verigene) different enteric pathogens; single sample can be tested	Expensive equipment and kit format	2 h	Biofire Diagnostics Inc.; Nanosphere Inc.; tests from other companies pending 510k clearance	Clinical laboratories

[a] Without nucleic acid extraction.

cell attachment factors for noroviruses (19). Expression of HBGAs on cell surfaces is affected by the ABO, Secretor, and Lewis genotypes. Because GII.4 viruses can bind a wider range of HBGAs than other genotypes, they are able to infect a larger susceptible population. Another mechanism which may explain the emergence of new variants is homologous recombination, with most breakpoints identified in the ORF1-ORF2 junction region. Intergenotype and intragenotype recombination is also widespread, suggesting that both escape from herd immunity and recombination are important factors that drive the emergence of novel GII.4 viruses (20).

DIAGNOSTIC METHODS

Although norovirus can be detected in rectal swabs and vomitus, whole-stool samples are the preferred clinical specimen for the detection of norovirus because they contain a higher quantity of virus. Until the cloning and sequencing of the Norwalk virus genome in 1990 (21) followed by the development and application of the first RT-PCR assays for norovirus, electron microscopy (EM) was the only method to detect the virus. Initially named Norwalk-like viruses or small-round structured viruses, based on their morphology in EM, this group of viruses is now officially known as noroviruses, with Norwalk virus as its prototype. Although EM can also visualize other established gastroenteritis viruses such as rotaviruses, adenoviruses, astroviruses, and sapoviruses, the method is costly and insensitive and therefore not widely available in diagnostic microbiology laboratories.

Because the rapid spread of norovirus is a major public health issue, rapid laboratory diagnosis is essential to assist implementation of appropriate control measures to reduce the spread of the virus and the magnitude of outbreaks. Hence, a simple rapid norovirus test would be an attractive alternative to more technically demanding assays such as enzyme immunoassays (EIAs) and reverse transcriptase PCR (Table 1). Immunochromatographic (ICG) lateral flow assays do not require specialized laboratory equipment and are designed for rapid (15-min) testing of individual samples. In a recent evaluation of 4 norovirus ICG tests, using a comprehensive panel of a wide variety of norovirus genotypes, the specificity of all tests was 100%. However, the overall sensitivity ranged from 35% to 52% and was strongly genogroup dependent, as the sensitivities ranged from 17% to 52% for GI strains to 59% to 78% for the predominant GII.4 viruses (22). These results were significantly lower than the sensitivities reported by other investigators as well as by the different manufacturers of the ICG kits, suggesting that robust evaluation of norovirus test requires validation with a norovirus stool panel that includes a wide variety of different GI and GII genotypes (22).

The development of a broadly reactive EIA for noroviruses has been challenging because of the number of antigenically distinct

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

TABLE 2 Norovirus genogroups and genotypes as determined by phylogeny-based cluster analysis of capsid protein VP1, partial capsid nucleotide region (region C or region D), and RNA polymerase region (POL genotype)

VP1 genotype[a]	Region C/region D genotype[b]	Reference strain[c]	GenBank accession no.	POL genotype	Reference strain	GenBank accession no.
GI.1	GI.1	GI/Hu/US/1968/GI.1/Norwalk	M87661	GI.P1	GI/Hu/US/1968/GI.P1/Norwalk	M87661
GI.2	GI.2	GI/Hu/GB/1991/GI.2/Southampton	L07418	GI.P2	GI/Hu/GB/1991/GI.P2/Southampton	L07418
GI.3	GI.3a	GI/Hu/SA/1990/GI.3/DesertShield395	U04469	GI.P3	GI/Hu/US/1998/GI.P3/VA98115	AY038598
	GI.3b	GI/Hu/NO/1995/GI.3/Stavanger	AF145709	GI.P4	GI/Hu/JP/1987/GI.P4/Chiba407	AB042808
	GI.3c	GI/Hu/JP/1979/GI.3/Otofuke	AB187514	GI.P5	GI/Hu/SE/2005/GI.P5/07_1	EU007765[d]
	GI.3d	GI/Hu/JP/1999/GI.3/Akabane	EF547396	GI.P6	GI/Hu/DE/1997/GI.P6/BS5(Hesse)	AF093797
GI.4	GI.4	GI/Hu/JP/1987/GI.4/Chiba407	AB042808	GI.P7	GI/Hu/SE/2008/GI.P7/Lilla Edet	JN603251
GI.5	GI.5a	GI/Hu/GB/1989/GI.5/Musgrove	AJ277614	GI.P8	GI/Hu/US/2008/GI.P8/890321	GU299761
	GI.5b	GI/Hu/JP/1999/GI.5/SzUG1	AB039774	GI.P9	GI/Hu/FR/2004/GI.P9/Chatellerault709	EF529737
GI.6	GI 6a	GI/Hu/DE/1997/GI.6/BS5(Hesse)	AJ277615	GI.Pa	GI/Hu/SA/1990/GI.Pa/DesertShield	U04469
	GI.6b	GI/Hu/GB/1995/GI.6/Sindlesham	AF093797	GI.Pb	GI/Hu/JP/2002/GI.Pb/WUG1	AB081723
GI.7	GI.7a	GI/Hu/GB/1994/GI.7/Winchester	AJ277609	GI.Pc	GI/Hu/JP/2000/GI.Pc/SzUG1	AB039774
	GI.7b	GI/Hu/US/2010/GI.7/Providence	JN899243	GI.Pd	GI/Hu/FR/2003/GI.Pd/Vesoul576	EF529738
	GI.7c	GI/Hu/JP/2003/GI.7/Chiba030100	AJ844469	GI.Pf	GI/Hu/JP/1979/GI.Pf/Otofuke	AB187514
GI.8	GI.8	GI/Hu/US/2001/GI.8/Boxer	AF538679	GII.P1	GII/Hu/US/1971/GII.P1/Hawaii	U07611
GI.9	GI.9	GI/Hu/CA/2004/GI.9/Vancouver730	HQ637267	GII.P2	GII/Hu/GB/1994/GII.P2/Melksham	X81879
GII.1	GII.1	GII/Hu/US/1971/GII.1/Hawaii	U07611	GII.P3	GII/Hu/CA/1991/GII.P3/Toronto	U02030
GII.2	GII.2	GII/Hu/GB/1994/GII.2/Melksham	X81879	GII.P4	GII/Hu/GB/1993/GII.P4/Bristol	X76716
GII.3	GII.3a	GII/Hu/CA/1991/GII.3/Toronto	U02030	GII.P5	GII/Hu/HU/1999/GII.P5/MOH	AF397156
	GII.3b	GII/Hu/AR/1999/GII.3/Arg320	AF190817	GII.P6	GII/Hu/JP/2002/GII.P6/Saitama U16	AB039778
	GII.3c	GII/Hu/NL/2006/GII.3/Rotterdam	AB385626	GII.P7	GII/Hu/JP/2002/GII.P7/Saitama U4	AB039777
GII.4 Bristol	GII.4	GII/Hu/GB/1993/GII.4/Bristol	X76716	GII.P8	GII/Hu/JP/2002/GII.P8/Saitama U25	AB039780
GII.4 New Orleans	GII.4 New Orleans	GII/Hu/US/2009/GII.4/NewOrleans	GU445325	GII.P11	GII/Po/US/1997/GII.P11/Sw918	AB074893
	GII.4 Apeldoorn	GII/Hu/NL/2007/GII.4/Apeldoorn	AB445395	GII.P12	GII/Hu/JP/2005/GII.P12/Sakai/04-179	AB220922
	GII.4 NSW001P	GII/Hu/AU/2008/GII.4/NSW001P	GQ845367	GII.P13	GII/Hu/FR/2004/GII.P13/Briancon870	EF529741
GII.4 Sydney	GII.4 Sydney	GII/Hu/AU/2012/GII.4/Sydney	JX459908	GII.P15	GII/Hu/JP/2006/GII.P15/Hiroshima66	AB360387
GII.5	GII.5	GII/Hu/GB/1990/GII.5/Hillingdon	AJ277607	GII.P16	GII/Hu/DE/2000/GII.P16/Neustrelitz260	AY772730
GII.6	GII.6a	GII/Hu/GB/1990/GII.6/Seacroft	AJ277620	GII.P18	GII/Po/US/2003/GII.P18/OH-QW101	AY823304
	GII.6b	GII/Hu/US/1994/GII.6/Miami292	AF414410	GII.P20	GII/Hu/GE/2005/GII.P20/Leverkusen267	EU424333
	GII.6c	GII/Hu/JP/2008/GII.6/Shizuoka	HM633213	GII.P21	GII/Hu/FR/2004/GII.P21/Pont de Roide673	AY682549
GII.7	GII.7	GII/Hu/GB/1990/GII.7/Leeds	AJ277608	GII.P22	GII/Hu/JP/2003/GII.P22/Hokkaido133	AB212306
GII.8	GII.8	GII/Hu/NL/1998/GII.8/Amsterdam	AF195848	GII.Pa	GII/Hu/JP/2004/GII.Pa/SN2000JA	AB190457
GII.9	GII.9	GII/Hu/US/1997/GII.9/VA97207	AY038599	GII.Pc	GII/Hu/US/1976/GII.Pc/SnowMountain	AY134748
GII.10	GII.10	GII/Hu/DE/2000/GII.10/Erfurt546	AF427118	GII.Pe	GII/Hu/JP/2007/GII.Pe/OC07138	AB434770

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

GII.11	GII.11	GII/Po/JP/1997/GII.11/Sw918	AB074893	GII.Pf	GII/Hu/FR/1999/GII.Pf/S63	AY682550
GII.12	GII.12	GII/Hu/GB/1990/GII.12/Wortley	AJ277618	GII.Pg	GII/Hu/AU/1983/GII.Pg/Goulburn Valley	DQ379714
GII.13	GII.13	GII/Hu/US/1998/GII.13/Fayetteville	AY113106	GII.Ph	GII/Hu/JP/1997/GII.Ph/OC97007	AB089882
GII.14	GII.14	GII/Hu/US/1999/GII.14/M7	AY130761	GII.Pj	GII/Hu/GR/1997/GII.Pj/E3	AY682552
GII.15	GII.15	GII/Hu/US/1999/GII.15/J23	AY130762	GII.Pk	GII/Hu/JP/1996/GII.Pk/OC96065	AF315813
GII.16	GII.16	GII/Hu/US/1999/GII.16/Tiffin	AY502010	GII.Pm	GII/Hu/IN/2006/GII.Pm/PunePC24	EU921353
GII.17	GII.17	GII/Hu/US/2002/GII.17/CS-E1	AY502009	GII.Pn	GII/Hu/CN/2007/GII.Pn/Beijing53931	GQ856469
GII.18	GII.18	GII/Po/US/2003/GII.18/OH-QW101	AY823304			
GII.19	GII.19	GII/Po/US/2003/GII.19/OH-QW170	AY823306			
GII.20	GII.20	GII/Hu/DE/2002/GII.20/Luckenwalde591	EU373815			
GII.21	GII.21	GII/Hu/IQ/2002/GII.21/IF1998	AY675554			
GII.22	GII.22	GII/Hu/JP/2003/GII.22/Yuri	AB083780			
GIII.1	N/A	GIII/Bo/DE/1980/GIII.1/Jena	AJ011099			
GIII.2	N/A	GIII/Bo/GB/1976/GIII.2/Newbury	AF097917			
GIII.3	N/A	GIII/Ov/NZ/2007/GIII.3/Norsewood30	EU193658			
GIV.1	N/A	GIV/Hu/NL/1998/GIV.1/Alphatron	AF195847			
GIV.2	N/A	GIV/Ca/IT/2006/GIV.2/Pistoia	EF450827			
GV.1	N/A	GV/Mu/US/2002/GV.1/MNV-1	AY228235			
GV.2	N/A	GV/Rn/HK/2011/GV.2/HKU_CT2	JX486101			
GVI.1	N/A	GVI/Ca/IT/2007/GVI.1/Bari91	FJ875027			
GVI.2	N/A	GVI/Ca/PT/2007/GVI.2/Viseu	GQ443611			
GVII	N/A	GVII/Ca/HK/2007/GVII/026F	FJ692500			

[a] Genotypes based on phylogenetic clustering of complete VP1 amino acids (13).
[b] Genotypes based on partial capsid sequences (region C and region D) as used by CaliciNet surveillance network (27). N/A, not available.
[c] Country abbreviations are as follows: AR, Argentina; AU, Australia; CA, Canada; CN, China; DE, Germany; FR, France; GB, United Kingdom; GR, Greece; HK, Hong Kong; HU, Hungary; IQ, Iraq; IN, India; JP, Japan; NL, Netherlands; NZ, New Zealand; NO, Norway; PT, Portugal; SA, Saudi Arabia; SE, Sweden; US, United States.
[d] This strain is 100% identical to the actual reference strain, which is pending GenBank submission.

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

humans norovirus genotypes ($n = 29$) and the antigenic drift of certain strains (e.g., GII.4) over time. Although genogroup-specific monoclonal antibodies have been described, most commercial kits, including the IDEIA Norovirus EIA (Oxoid, Hampshire, United Kingdom), SRSV (II)-AD (Denka Seiken Co. Ltd., Tokyo, Japan), and RIDASCREEN (r-Biopharm AG, Darmstadt, Germany), include combinations of several cross-reactive monoclonal and polyclonal antibodies. The sensitivity of these kits is typically <70%, while the specificity is usually >90%, depending on the diagnostic goal (outbreak or sporadic cases), the number of samples tested per outbreak, and the time after the onset of symptoms that clinical samples were collected. The general scientific consensus is that EIA may be useful for rapid screening of multiple fecal samples collected during an outbreak of acute gastroenteritis for norovirus but, because of the low sensitivity, caution should be exercised in interpreting test results from sporadic cases (23).

In the mid-1990s, the first conventional or endpoint RT-PCR assays were developed, targeting a relatively conserved small region of the RNA polymerase (POL) gene in ORF1 (region A). With the increasing number of sequences that became available during those early years, those assays were rapidly replaced by second-generation assays that proved to be more broadly reactive and able to detect the majority of the circulating norovirus strains. One of those early assays is, in a slightly modified format, still being used successfully for the detection and typing of noroviruses (24). Increased specificity and sensitivity are accomplished by the use of real-time RT-quantitative PCR assays (RT-qPCR) that do not require agarose gel analysis and subsequent confirmation and, in most protocols, use fluorescently labeled oligonucleotide probes. One-step RT-qPCR assays, in which both reverse transcription and cDNA amplification are performed in a single reaction, require less sample handling and therefore decrease the risk of cross-contamination, making them a preferred format in clinical laboratories. Because only one small region of the norovirus genome is sufficiently conserved for the development of genogroup-specific oligonucleotide primers and probes (25), most of the reported norovirus RT-qPCR assays target this ORF1-ORF2 junction region (26, 27). And although no commercial standalone norovirus RT-qPCR assay has yet been FDA cleared, in recent years these assays have become the gold standard for the rapid and sensitive detection of norovirus in clinical (stool, vomitus, serum) samples as well as in food, water, and environmental samples. Increasingly, RT-qPCR assays include an internal extraction control to reduce false-negative results and are able to simultaneously detect GI and GII strains (28) or GI, GII, and GIV strains (29). In addition to the high analytical sensitivity, RT-qPCR assays can also be used to determine the amount of nucleic acid in a sample in a semiquantitative way as a proxy to determine the viral load. Patients with higher viral loads have been reported to excrete the virus longer, and data from several studies suggest that GII viruses (i.e., GII.4) are shed in larger amounts than GI viruses (30).

A significant number of patients excrete the virus 3 weeks after clinical symptoms have disappeared, and noroviruses are also frequently detected in fecal samples from asymptomatic patients, in particular, in children under the age of 5. Hence, virus detection by RT-qPCR does not always correlate with clinical norovirus disease but assessment of a possible difference in viral load in samples from clinical and asymptomatic cases may be a helpful tool to assess a causal relationship with clinical symptoms. In a study in the United Kingdom, higher viral loads were found in norovirus-positive cases than in asymptomatic controls and a clinically significant cutoff value of 31 cycles for all ages resulted in a sensitivity of 72% (31). However, in other studies, including studies in low-income countries, norovirus was as commonly detected in stools from cases with moderate to severe diarrhea as in those from healthy controls and was present in similar viral loads (reference 32 and unpublished data). This makes interpretation of positive RT-qPCR results, particularly those determined from samples with low viral loads (high threshold cycle [C_T] values), a challenge. Additional data from studies of considerable sample size are required to determine robust C_T cutoff values to interpret norovirus RT-qPCR results. Such cutoff values may depend on variables such as the sample collection date, PCR platform, reagent or kit used, and study population (e.g., outbreak versus sporadic samples). Alternatively, data from outbreak studies in which multiple samples have been collected from norovirus-positive patients after they have become asymptomatic may help in establishing a clinically relevant cutoff value.

In recent years, several different multi-gastrointestinal-pathogen diagnostic platforms have been developed for the simultaneous detection of pathogenic enteric viruses, bacteria, and parasites (Table 1). The xTAG GPP (Luminex Corporation, Toronto, Canada), FilmArray GI Panel (BioFire Diagnostics Inc., Salt Lake City, UT, USA), and Verigene Enteric Pathogens Test (EP) (Nanosphere, Northbrook, IL, USA) platforms have recently been FDA cleared and currently provide the most comprehensive commercial multiplex molecular diagnostic tests available for gastroenteritis diagnosis. The FDA-cleared version of the xTAG GPP platform simultaneously detects and identifies norovirus GI and GII, rotavirus group A, 7 bacterial species, and 2 parasite species (33), while the FilmArray GI Panel detects 23 enteric pathogens, including norovirus GI and GII, rotavirus group A, group F adenovirus, sapovirus, and astrovirus, 14 bacterial species, and 4 parasite species. The Verigene EP assay detects 5 bacterial species, 2 Shiga toxins, rotavirus, and norovirus. The Biofire and Luminex platforms are able to distinguish between GI and GII noroviruses. However, there are significant differences between these tests, including workflow and throughput differences (Table 1). The xTAG GPP can complete testing of 24 samples within 5 h, but this does not include preparation and extraction of the samples. In contrast, the FilmArray and Verigene systems have a turnaround time from unprocessed sample to results of 2 h, with minimal hands-on time. The drawback of the FilmArray and Verigene systems in the current formats is their low throughput, as only a single sample can be processed on the instrument at one time, which may not be an issue for clinical laboratories but limits the overall utility of the test in laboratories with moderate to high throughput. Another challenge of these multipathogen systems is that of data interpretation, specifically with high numbers of mixed infections and the lack of quantitative data to determine which pathogen is responsible for the gastrointestinal disease (34).

If no laboratory diagnosis can be performed (e.g., when no specimens are available for testing), norovirus infections can also be detected on the basis of the clinical and epidemiological profile, which has been used successfully to differentiate norovirus causing gastroenteritis from other causes of enteric disease. These Kaplan criteria (35) are based on (i) a mean duration of illness of between 12 and 60 h, (ii) a mean incubation period of 24 to 48 h, (iii) vomiting in >50% of patients, and (iv) the absence of bacte-

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

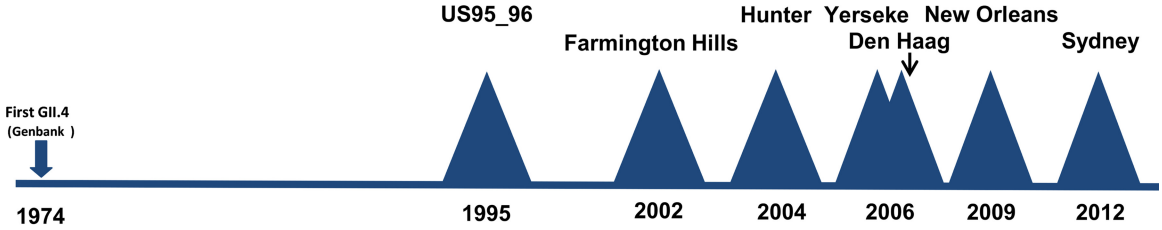


FIG 2 GII.4 norovirus variants with a global distribution and the first season in which they emerged. New GII.4 variants emerge approximately every 2 to 3 years and replace the previously predominant strains. They include US95_96 in 1995, Farmington Hills in 2002, Hunter in 2004, Yerseke in 2006, Den Haag in 2006, New Orleans in 2009, and Sydney in 2012.

rial pathogens detected in stool specimens. The criteria are highly specific (99%) and moderately sensitive (68%) for foodborne outbreaks but may not be valid for hospital outbreaks, where the duration of symptoms can be longer than 72 h.

GENOTYPING

Noroviruses are classified into genogroups and genotypes based on amino acid diversity in the complete VP1 protein, but, as recombination in the ORF1-ORF2 junction region is common and as some capsid genotypes seem to be more prone to recombination than others, a dual-nomenclature system has been proposed using both the RNA polymerase (POL) region in ORF1 and VP1 sequences (13) (Table 2). Currently, 9 genotypes in GI, 22 in GII, 2 in GIII, 2 in GIV, 1 in GV, 2 in GVI, and 1 in the tentative new GVII have been recognized on the basis of complete VP1 amino acids (Fig. 1). The nomenclature system includes information on genogroup, genotype, and, for GII.4 strains, variant type. For example, if both POL and capsid (CAP) sequences are known, the strain name should be written as follows: norovirus GII/Hu/US/2010/GII.P12-GII.12/HS206. When only CAP sequences are available, the strain should be written as follows: norovirus GII/Hu/AU/2012/GII.4 Sydney/Melbourne456.

Because sequencing of the complete VP1 gene is currently not a routine procedure, nucleotide sequences of relatively small regions of ORF1 (POL or region A) or ORF2 (CAP or regions C and D) of the norovirus genome are used to genotype strains. Region C assays are in general more robust because the lower (40°C) annealing temperature required for the region D assays increases the likelihood of nonspecific amplification and because region D is located in a more variable part of ORF2. As determined on the basis of nucleotide sequence diversity in region C and region D, several genotypes consist of up to 4 different subclusters (e.g., GI.3a to GI.3d); therefore, reference sequences representative of each subcluster are required for correct typing of these strains (Table 2). An online norovirus typing tool is available for both polymerase and capsid typing (36).

GII viruses, in particular, GII.4 viruses, are responsible for the majority of the norovirus outbreaks in people of all ages worldwide, whereas GI strains are more often detected in foodborne and waterborne outbreaks. For example, the GI.6 virus that emerged in 2012 was more often associated with foodborne disease outbreaks than the GII.4 viruses, which are strongly associated with person-to-person transmission and outbreaks in health care settings, resulting in an increased risk of more-severe disease outcomes such as hospitalization and death compared to other GI and GII viruses (37). GII.4 viruses have an epidemiology different those of other GI and GII genotypes. Since the mid-1990s, 7 different GII.4 variants have successively emerged every 2 to 3 years, replacing previous dominant variants, and most of them have produced global epidemics of gastroenteritis. The first reported GII.4 pandemic (caused by GII.4 US95_96) occurred in 1995, followed by the emergence of GII.4 Farmington Hills in 2002, GII.4 Hunter in 2004, GII.4 Yerseke and GII.4 Den Haag in 2006, GII.4 New Orleans in 2009, and GII.4 Sydney in 2012 (Fig. 2). Although media coverage often suggests otherwise, studies in the United States have shown that neither the emergence of GII.4 New Orleans in 2009 nor that of GII.4 Sydney in 2012 led to an increase in norovirus activity compared to previous years. These findings underscore the importance of conducting well-designed studies to better understand the contributions that individual genotypes may make to norovirus disease burden. Between 2009 and 2013, several non-GII.4 strains (GII.12, GII.1, GI.6) have emerged that cocirculated with the predominant GII.4 viruses and caused 11% to 15% of all outbreaks, but each strain did not circulate longer than one norovirus season (16). Genotype distribution in sporadic norovirus disease usually follows the same trends as in outbreaks (2), although rare genotypes are often reported in children under 5 years of age. Continuous norovirus outbreak surveillance through surveillance networks such as NoroNet and CaliciNet will be important to identify changing trends in genotype distribution and identify emerging new strains.

FUTURE PERSPECTIVES

Noroviruses are the leading cause of epidemic and sporadic cases of acute gastroenteritis worldwide and a leading cause of foodborne disease. Therefore, rapid laboratory diagnosis is a critical tool to guide controlling norovirus outbreaks by choosing the most appropriate intervention and control practices such as enhanced cleaning and disinfection protocols, isolation, grouping patients based on symptoms, exclusion of symptomatic staff members or food handlers, or, ultimately, closing of units in hospitals (38). Over the last decade, significant progress has been made in the development of diagnostic methods for the routine detection of human noroviruses. RT-qPCR assays have become the gold standard for norovirus detection in most public health and research laboratories and are increasingly commercially available. Continued improvement of rapid, sensitive, and broadly reactive point-of-care assays, such as ICG assays, will be required to allow simple and reliable norovirus diagnosis where no laboratory facilities are available. Use of the multi-gastrointestinal-pathogen molecular platforms that are now available for the rapid detection of a suite of different enteric pathogens, including norovirus, in a single sample will become routine in many clinical laboratories over the next couple of years.

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

Recent advances in nucleic acid sequencing technologies, such as "next-generation" sequencing (NGS), have opened new perspectives for research and diagnostic applications because of the high speed and throughput of data generation. NGS has been applied for the discovery of novel viruses and the characterization of viral communities as well as whole-viral-genome sequencing and detection of the viral genome variability of RNA viruses. Although challenges remain, including difficulties in sample preparation and high cost, NGS is a potentially powerful method for the rapid identification, and characterization of any infectious agent, including norovirus, directly from stool could assist in infection control of outbreaks.

Genotyping of norovirus strains is important, as certain genotypes are more often associated with foodborne transmission whereas others (e.g., GII.4) have led to more-severe disease outcomes. Standardized genotyping as performed by surveillance networks such as CaliciNet and NoroNet will make it easier to identify new emerging strains or common-source outbreaks and provide useful information on the distribution of strains in different populations, which is important for norovirus vaccine formulations (39).

ACKNOWLEDGMENTS

Many thanks to my colleagues at CDC and the CaliciNet surveillance network for their continued efforts to improve our understanding of the public health burden of norovirus, to Everardo Vega for help with Fig. 1, and to Harry Vennema (RIVM) for sharing polymerase gene reference sequences used in NoroNet.

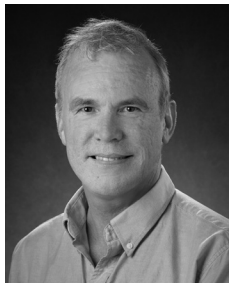
The findings and conclusions in this article are mine and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

REFERENCES

1. **Glass RI, Parashar UD, Estes MK.** 2009. Norovirus gastroenteritis. N Engl J Med **361:**1776–1785. http://dx.doi.org/10.1056/NEJMra0804575.
2. **Payne DC, Vinjé J, Szilagyi PG, Edwards KM, Staat MA, Weinberg GA, Hall CB, Chappell J, Bernstein DI, Curns AT, Wikswo M, Shirley SH, Hall AJ, Lopman B, Parashar UD.** 2013. Norovirus and medically attended gastroenteritis in U.S. children. N Engl J Med **368:**1121–1130. http://dx.doi.org/10.1056/NEJMsa1206589.
3. **Hall AJ, Wikswo ME, Manikonda K, Roberts VA, Yoder JS, Gould LH.** 2013. Acute gastroenteritis surveillance through the National Outbreak Reporting System, United States. Emerg Infect Dis **19:**1305–1309. http://dx.doi.org/10.3201/eid1908.130482.
4. **Hall AJ, Lopman BA, Payne DC, Patel MM, Gastañaduy PA, Vinjé J, Parashar UD.** 2013. Norovirus disease in the United States. Emerg Infect Dis **19:**1198–1205. http://dx.doi.org/10.3201/eid1908.130465.
5. **Koo HL, Neill FH, Estes MK, Munoz FM, Cameron A, Dupont HL, Atmar RL.** 2013. Noroviruses: the most common pediatric viral enteric pathogen at a large university hospital after introduction of rotavirus vaccination. J Pediatric Infect Dis Soc **2:**57–60. http://dx.doi.org/10.1093/jpids/pis070.
6. **Bok K, Green KY.** 2012. Norovirus gastroenteritis in immunocompromised patients. N Engl J Med **367:**2126–2132. http://dx.doi.org/10.1056/NEJMra1207742.
7. **Wingfield T, Gallimore CI, Xerry J, Gray JJ, Klapper P, Guiver M, Blanchard TJ.** 2010. Chronic norovirus infection in an HIV-positive patient with persistent diarrhoea: a novel cause. J Clin Virol **49:**219–222. http://dx.doi.org/10.1016/j.jcv.2010.07.025.
8. **Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, Ramani S, Hill H, Ferreira J, Graham DY.** 2014. Determination of the 50% human infectious dose for Norwalk virus. J Infect Dis **209:**1016–1022. http://dx.doi.org/10.1093/infdis/jit620.
9. **Prasad BV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK.** 1999. X-ray crystallographic structure of the Norwalk virus capsid. Science **286:**287–290. http://dx.doi.org/10.1126/science.286.5438.287.
10. **Vongpunsawad S, Venkataram Prasad BV, Estes MK.** 2013. Norwalk virus minor capsid protein VP2 associates within the VP1 shell domain. J Virol **87:**4818–4825. http://dx.doi.org/10.1128/JVI.03508-12.
11. **Green K.** 2013. Caliciviridae: the noroviruses, p 583–609. *In* Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B (ed), Fields virology, 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
12. **Tse H, Lau SK, Chan WM, Choi GK, Woo PC, Yuen KY.** 2012. Complete genome sequences of novel canine noroviruses in Hong Kong. J Virol **86:**9531–9532. http://dx.doi.org/10.1128/JVI.01312-12.
13. **Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M.** 2013. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol **158:**2059–2068. http://dx.doi.org/10.1007/s00705-013-1708-5.
14. **Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS.** 2006. Norovirus classification and proposed strain nomenclature. Virology **346:**312–323. http://dx.doi.org/10.1016/j.virol.2005.11.015.
15. **Martella V, Decaro N, Lorusso E, Radogna A, Moschidou P, Amorisco F, Lucente MS, Desario C, Mari V, Elia G, Banyai K, Carmichael LE, Buonavoglia C.** 2009. Genetic heterogeneity and recombination in canine noroviruses. J Virol **83:**11391–11396. http://dx.doi.org/10.1128/JVI.01385-09.
16. **Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinjé J.** 2014. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol **52:**147–155. http://dx.doi.org/10.1128/JCM.02680-13.
17. **Siebenga JJ, Vennema H, Zheng DP, Vinjé J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M.** 2009. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001–2007. J Infect Dis **200:**802–812. http://dx.doi.org/10.1086/605127.
18. **Leshem E, Wikswo M, Barclay L, Brandt E, Storm W, Salehi E, Desalvo T, Davis T, Saupe A, Dobbins G, Booth HA, Biggs C, Garman K, Woron AM, Parashar UD, Vinjé J, Hall AJ.** 2013. Effects and clinical significance of GII.4 Sydney norovirus, United States, 2012–2013. Emerg Infect Dis **19:**1231–1238. http://dx.doi.org/10.3201/eid1908.130458.
19. **Lindesmith LC, Beltramello M, Donaldson EF, Corti D, Swanstrom J, Debbink K, Lanzavecchia A, Baric RS.** 2012. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. PLoS Pathog **8:**e1002705. http://dx.doi.org/10.1371/journal.ppat.1002705.
20. **Eden JS, Tanaka MM, Boni MF, Rawlinson WD, White PA.** 2013. Recombination within the pandemic norovirus GII.4 lineage. J Virol **87:**6270–6282. http://dx.doi.org/10.1128/JVI.03464-12.
21. **Xi JN, Graham DY, Wang KN, Estes MK.** 1990. Norwalk virus genome cloning and characterization. Science **250:**1580–1583. http://dx.doi.org/10.1126/science.2177224.
22. **Ambert-Balay K, Pothier P.** 2013. Evaluation of 4 immunochromatographic tests for rapid detection of norovirus in faecal samples. J Clin Virol **56:**194–198. http://dx.doi.org/10.1016/j.jcv.2012.11.001.
23. **Costantini V, Grenz L, Fritzinger A, Lewis D, Biggs C, Hale A, Vinjé J.** 2010. Diagnostic accuracy and analytical sensitivity of IDEIA Norovirus assay for routine screening of human norovirus. J Clin Microbiol **48:**2770–2778. http://dx.doi.org/10.1128/JCM.00654-10.
24. **Vennema H, de Bruin E, Koopmans M.** 2002. Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction. J Clin Virol **25:**233–235. http://dx.doi.org/10.1016/S1386-6532(02)00126-9.
25. **Katayama K, Shirato-Horikoshi H, Kojima S, Kageyama T, Oka T, Hoshino F, Fukushi S, Shinohara M, Uchida K, Suzuki Y, Gojobori T, Takeda N.** 2002. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. Virology **299:**225–239. http://dx.doi.org/10.1006/viro.2002.1568.
26. **Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K.** 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol **41:**1548–1557. http://dx.doi.org/10.1128/JCM.41.4.1548-1557.2003.
27. **Vega E, Barclay L, Gregoricus N, Williams K, Lee D, Vinjé J.** 2011. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. Emerg Infect Dis **17:**1389–1395. http://dx.doi.org/10.3201/eid1708.101837.

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library

28. **Rolfe KJ, Parmar S, Mururi D, Wreghitt TG, Jalal H, Zhang H, Curran MD.** 2007. An internally controlled, one-step, real-time RT-PCR assay for norovirus detection and genogrouping. J Clin Virol **39:**318–321. http://dx.doi.org/10.1016/j.jcv.2007.05.005.
29. **Miura T, Parnaudeau S, Grodzki M, Okabe S, Atmar RL, Le Guyader FS.** 2013. Environmental detection of genogroup I, II, and IV noroviruses by using a generic real-time reverse transcription-PCR assay. Appl Environ Microbiol **79:**6585–6592. http://dx.doi.org/10.1128/AEM.02112-13.
30. **Chan MC, Sung JJ, Lam RK, Chan PK, Lee NL, Lai RW, Leung WK.** 2006. Fecal viral load and norovirus-associated gastroenteritis. Emerg Infect Dis **12:**1278–1280. http://dx.doi.org/10.3201/eid1208.060081.
31. **Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D, Gray J.** 2009. Diagnosing norovirus-associated infectious intestinal disease using viral load. BMC Infect Dis **9:**63. http://dx.doi.org/10.1186/1471-2334-9-63.
32. **Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM.** 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet **382:**209–222. http://dx.doi.org/10.1016/S0140-6736(13)60844-2.
33. **Navidad JF, Griswold DJ, Gradus MS, Bhattacharyya S.** 2013. Evaluation of Luminex xTAG gastrointestinal pathogen analyte-specific reagents for high-throughput, simultaneous detection of bacteria, viruses, and parasites of clinical and public health importance. J Clin Microbiol **51:**3018–3024. http://dx.doi.org/10.1128/JCM.00896-13.
34. **Khare R, Espy MJ, Cebelinski E, Boxrud D, Sloan LM, Cunningham SA, Pritt BS, Patel R, Binnicker MJ.** 2014. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. J Clin Microbiol **52:**3667–3673. http://dx.doi.org/10.1128/JCM.01637-14.
35. **Kaplan JE, Gary GW, Baron RC, Singh N, Schonberger LB, Feldman R, Greenberg HB.** 1982. Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann Intern Med **96:**756–761.
36. **Kroneman A, Vennema H, Deforche K, v d Avoort H, Peñaranda S, Oberste MS, Vinjé J, Koopmans M.** 2011. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol **51:**121–125. http://dx.doi.org/10.1016/j.jcv.2011.03.006.
37. **Desai R, Hembree CD, Handel A, Matthews JE, Dickey BW, McDonald S, Hall AJ, Parashar UD, Leon JS, Lopman B.** 2012. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. Clin Infect Dis **55:**189–193. http://dx.doi.org/10.1093/cid/cis372.
38. **Barclay L, Park GW, Vega E, Hall A, Parashar U, Vinjé J, Lopman B.** 2014. Infection control for norovirus. Clin Microbiol Infect **20:**731–740. http://dx.doi.org/10.1111/1469-0691.12674.
39. **Debbink K, Lindesmith LC, Baric RS.** 2014. The state of norovirus vaccines. Clin Infect Dis **58:**1746–1752. http://dx.doi.org/10.1093/cid/ciu120.



Jan Vinjé, Ph.D., is head of the National Calicivirus Laboratory and Director of CaliciNet at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA. Dr. Vinjé received his Ph.D. degree at the University of Utrecht, the Netherlands, in 1999. After receiving a postdoctoral fellowship and an appointment as research assistant professor at the University of North Carolina in Chapel Hill, he joined CDC in 2006. Over the past 10 years, he has served on several program advisory committees from several European research projects (FP6, FP7). He is serving as technical expert on the norovirus subcommittee of the National Advisory Committee on Microbiological Criteria for Foods and is a member of the International Committee on Taxonomy of Viruses study groups on *Caliciviridae* (chair as of 2014) and *Astroviridae*. He is currently a member of the editorial board of the *Journal of Clinical Microbiology* and associate editor of the journal *Food and Environmental Virology*, and he serves as an *ad hoc* reviewer for multiple high-impact journals. Dr. Vinjé has published over 100 peer-reviewed publications and several book chapters. His research interests include all aspects of viral gastrointestinal disease, including detection, characterization, and prevention and control of norovirus infections.

Downloaded from http://jcm.asm.org/ on April 6, 2018 by Stephen B. Thacker CDC Library