

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2016 ISBN 1595-689X VOL17 No. 3
AJCEM/1630 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2016 <http://dx.doi.org/10.4314/ajcem.v17i3.8>
AFR. J. CLN. EXPER. MICROBIOL. 17 (3): 205-212

PERFORMANCE CHARACTERISTICS OF ENZYME LINKED IMMUNOSORBENT ASSAY AND RAPID IMMUNOCHROMATOGRAPHIC TEST FOR ROUTINE SCREENING OF HUMAN NOROVIRUS

Sharaf⁽¹⁾, H E, Morsi⁽¹⁾, S S and Gerges⁽¹⁾, M A

⁽¹⁾Medical Microbiology & Immunology Department, Faculty of Medicine, Zagazig University, Egypt

ABSTRACT

Noroviruses (NoV) are identified as the major cause of epidemic and sporadic acute gastroenteritis. Controlling the spread of the disease needs early recognition of NoV. This study investigated the contribution of norovirus to sporadic cases of pediatric gastroenteritis in Zagazig University Hospitals and studied the performance characteristics of enzyme linked immunosorbent assay (EIA) and immunochromatographic (ICT) assay for their ability to detect NoV. Two hundred stool specimens were collected from pediatric patients with acute gastroenteritis. Samples were tested for Norovirus presence by reverse transcription PCR (RT-PCR), ICT kit and EIA. 27% of the samples showed the 338-bp portion of the RNA-dependent RNA polymerase (*RdRp*) gene of both Norovirus genogroups I and II by RT-PCR. The ICT assay showed high specificity (97.94%) and high sensitivity (85.18%). The EIA showed high specificity (93.8%) but low sensitivity (64.8%). In conclusion, the high detection rate of NoV as the cause of diarrhea in children reported in this study supports their addition in screenings to identify sporadic cases of acute gastroenteritis. The ICT and RIA Norovirus kits may be useful for rapid screening of stool samples from patients with acute gastroenteritis. However, RT-PCR should be considered for negative samples to be confirmed.

Key words: Norovirus, pediatric gastroenteritis, RNA-dependent RNA polymerase (*RdRp*) gene, enzyme linked immunosorbent assay, immunochromatographic assay, Sensitivity, Specificity.

Abbreviations: NoV, Noroviruses; EIA, enzyme linked immunosorbent assay; ICT, immunochromatographic; RT-PCR, reverse transcription PCR; *RdRp*, RNA-dependent RNA polymerase; ORFs, open reading frames.

CHARACTERISTIQUES DE PERFORMANCE DU DOSAGE IMMUNO - ENZYMATIQUE ET TEST IMMUNOCHROMATOGRAPHIQUE RAPIDE POUR LE DÉPISTAGE ROUTINE DU NOROVIRUS HUMAIN

HendElsayedSharaf¹, SamarSaeedMorsi¹, et MarianAsaadGerges¹;

¹Département de Microbiologie Médicale et Immunologie, Faculté de Médecine, l'Université de Zagazig.

RESUME

Les norovirus (NoV) sont identifiés comme la cause majeure d'épidémie et la gastro-entérite aiguë sporadique. Le contrôle de la propagation de la maladie a besoin de la reconnaissance précoce des norovirus. Cette étude a examiné la contribution des norovirus à des cas sporadiques de gastro-entérite pédiatrique dans les hôpitaux universitaires de Zagazig a étudié les caractéristiques de performance de dosage immuno - enzymatique (EIA) et dosage immunochromatographique (ICT) pour leur capacité. Deux cents des échantillons de selles ont été recueillies auprès des patients pédiatriques atteints de gastro-entérite aiguë. Les échantillons ont été testés pour la présence de norovirus par transcription inverse PCR (RT-PCR), kit d'ICT et EIA. 27% des échantillons a montré la partie 338 bp de l'ARN - le gène l'ARN polymérase dépendante (*RdRd*) des deux génogroupes de norovirus I et II par RT - PCR. Le dosage de ICT a montré une grande spécificité (97,94%) et une sensibilité (85,18%) élevée. L'EIA a montré une grande spécificité (93,8%), mais une faible sensibilité. (64,8%). En conclusion, le taux de détection élevé de norovirus comme la cause de la diarrhée chez les enfants rapporté dans cette étude prend en charge leur addition dans les dépistages pour identifier des cas sporadiques de gastro-entérite aiguë. Les kits de ICT et RIA de norovirus peut - être utiles pour le dépistage rapide des échantillons de selle de patients atteints le gastro-entérite aiguë. Cependant, la RT-PCR devrait être considérée pour les échantillons négatifs à confirmer.

Mots clés: Norovirus, gastro-entérite pédiatrique, le gène de l'ARN- l'ARN polymérase dépendante (*RdRd*), le dosage immuno enzymatique, dosage immunochromatographique, sensibilité, spécificité.

Abbréviations : NoV.: norovirus; EIA: Dosage immuno - enzymatique; ICT :Immunochromatographique; RT -PCR: Transcriptions inverse PCR; *RdRd* : ARN dépendant ARN polymérase; ORFs :Cadre de lecture ouvert.

INTRODUCTION

Norovirus (NoV) is an important cause of acute nonbacterial gastroenteritis in children and adults worldwide. NoV is classified in the *Calicivirus* family which includes vesivirus, lagovirus, and sapovirus. The genome of caliciviruses is single-stranded, positive-sense RNA, 7.5 kb approximately, which are arranged into three open reading frames (ORFs) that encode the nonstructural proteins (ORF1), a capsid protein (ORF2), and a small basic protein (ORF3) (1).

Improved diagnostics of NoV have increased its identification to become the leading cause of epidemic and sporadic gastroenteritis in people of all age groups worldwide (2). Most of NoV outbreaks take place in hospitals, where the virus is mainly transmitted from one person to another. In addition, noroviruses have also been diagnosed in over 58% of the announced food borne outbreaks in which an etiologic agent was identified (3).

NoV, except of murine strains, cannot be cultivated *in vitro*, which makes their classification into distinct serotypes very difficult. Consequently, they are genetically classified into 6 recognised geno-groups (GI to GVI) (4).

Early recognition of NoV is important to decrease the transmission of disease in outbreaks (5). Norwalk virus was discovered in 1972 by electron microscopy, then cloning and sequencing in 1990 occur. Diagnosis of NoV was done by conventional reverse RT-PCR assay in the mid-1990s followed by real-time RT-PCR (rRT-PCR) assays, which is now the method used for diagnosis of NoV in most clinical laboratories (6). The role of NoV as an important cause of both epidemic and sporadic gastroenteritis were better understood using molecular techniques and sequence analysis (7). Wide differences within this genus were detected using genomic analysis. These diversity are present even in the (*RdRp*) gene, which is supposed to be conserved. *RdRp* gene is used not only for the identification of gastroenteritis caused by NoVs, but also for molecular and epidemiological studies of calicivirus infections because of its lower diversity (8).

Rapid detection of NoV using various kits have been established lately and are commercially available such as enzyme EIAs and ICT tests. Large-scale clinical and epidemiological studies can use these kits. These kits require antibodies that cross react between NoV genogroups to overcome the high antigenic diversity of NoVs and help to detect new strains. The usefulness of these kits for routine screening of samples should be investigated because the sensitivity and specificity of NoV diagnostic kits vary with the diagnostic goal (outbreak or sporadic cases) and test design (6).

The aim of the present study was to investigate the contribution of NoV to sporadic cases of pediatric gastroenteritis in Zagazig University Hospitals and to evaluate the IDEIA norovirus assay (Oxoid, UK) together with a second assay, RIDA quick Norovirus Test (R-BioPharm, Darmstadt, Germany), for their capability to detect NoV antigen in faecal samples by comparing the obtained results with RT-PCR results.

MATERIALS AND METHODS

Stool samples.

Two hundred stool specimens were collected at Zagazig University Hospitals, Egypt, from 1-month-old to 12-year-old patients who visited the clinics for acute gastroenteritis from November 2014 to June 2015. Specimens were collected within 72 h following the onset of symptoms. Samples were routinely tested for the presence of bacterial infections and fecal leukocytes in the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University.

Sample preparation and RNA extraction

Stool suspensions (10%) were prepared in phosphate-buffered saline (pH 7.2 to 7.4) by standard methods as soon as they arrive to the laboratory. Isolation of RNA from 140 µl stool extracts was done using QIAamp microspin columns (viral RNA minikit; QIAGEN) according to the manufacturer's protocols. Aliquots containing the eluted RNA in 60 µl of elution buffer were stored at -20°C until testing. If the IDEIA Norovirus test was going to be done in a maximum of 3 days, specimens were stored at 2-8°C. Faecal specimens were kept at -20°C in case of longer storage.

RT-PCR

Testing stool specimens for NoV was conducted by nested RT-PCR, using the primer pair NV32 (5'-4226-4245 ATG AAT ATG AATGAA GAT GG-3') and NV36 (5'-4707-4726 ATT GGT CCT TCT GIT TTGTC-3') for first-round amplification and primer pair NV33 (5'-4280-4299 TACCAC TAT GAT GCA GAT TA-3') and NV35 (5'-4617-4636 GTT GAC ACAATC TCA TCA TC-3') for second-round amplification (9). These primers amplified the *RdRp* gene of both genogroups I and II with a final product of 338-bp band.

Five microliter of extracted RNA was added to a 15 µl mix consisting of 10 mM Tris-HCl (pH 8.3), 5 mM MgCl₂, 50 mM KCl, deoxynucleoside triphosphate at a concentration of 1 mM each, together with 20 U of avian myeloblastoma virus reverse transcriptase (Roche Applied Science, Mannheim, Germany). Reverse transcription cycles were conducted as follow: 10 min at 25°C, then 60 min at 42°C, finally, the reaction was stopped using denaturing the enzyme for 5 min at 98°C then cooling of the mixture down to 4°C was done. The resulting cDNA (2 µl) was added to the PCR mix (48 µl) in a 50 µl reaction mixture consisting of 10 mM Tris-HCl, 1.25 mM

MgCl₂, 50 mM KCl, 2.5 U of *Taq* polymerase (Roche Applied Science, Mannheim, Germany), 30 pmol each of the NV32 and NV36 primers and each deoxynucleoside triphosphate at a concentration of 125 μM. Amplification was done according to the following cycles: primary denaturation at 94°C for 60 s; 35 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, and extension at 72°C for 45 s; and a final extension at 72°C for 3 min. One microliter of the first PCR product was used in the nested PCR under similar reaction conditions with primers NV33 and NV35. Examination of final product was done by gel electrophoresis in 2% agarose gels containing ethidium bromide (8).

The RIDA QUICK Norovirus Test. (R-Biopharm, Darmstadt, Germany).

Rida quick Norovirus Test was used for antigen detection in stool suspension according to the manufacturer protocols. The Test which can determine GI and GII noroviruses in stool samples is a quick qualitative immunochromatographic test. It helps in the diagnosis of gastroenteritis and for the analysis of stool samples from pediatric and adult patients suspected to have gastroenteritis caused by NoV.

This assay employed both biotinylated and gold-labeled anti-norovirus antibodies for quick diagnosis. Immune complexes are formed between NoV and the gold-labeled anti-norovirus antibodies and migrate through the reaction membrane in positive samples. The migrating immune complexes are captured by streptavidin at the test line (T line) via the biotin joined to the anti-norovirus antibodies, and the T line is stained in red violet colour. Gold-labeled antibodies which is not bound in the complex migrate and captured at the control line (C line). In negative samples, the binding of gold-labeled immunocomplexes will occur only at the C line but not at the T line. Validation of the test is confirmed by the presence of a red C line.

The IDEIA Norovirus test (Oxoid, UK).

IEA was done to identify NoV antigen in 10% stool suspension according to the manufacturer protocols. The IDEIA Norovirus test detect GI and GII NoV utilising a combination of both GI and GII specific polyclonal and monoclonal antibodies fixed in a solid phase sandwich enzyme immunoassay. A mixture of genogroup 1 and genogroup 2 NoV specific monoclonal antibodies are coating microwells. Control or faecal suspension is added to the microwell and incubated simultaneously with a mixture of NoV specific GI and GII polyclonal and monoclonal antibodies conjugated to horseradish peroxidase. Capturing of Norovirus antigen present in the specimen takes place between solid phase antibodies and the enzyme conjugated antibodies. Microwells are washed with wash buffer to remove any unbound enzyme labeled antibodies and excess specimen after 60 minutes incubation at room temperature. After adding chromogen, the microwells is incubated for 30 minutes at room temperature. A colour change will occur in the presence of specifically bound enzyme labelled antibodies in the microwells, which is stopped by the addition of acid. The presence of NoV antigen in the specimen or control is indicated by colour intensity above background levels.

RESULTS

In this work 200 stool specimens were collected at Zagazig University Hospitals, Egypt, from 1-month-old to 12-year-old patients who visited the clinics with symptoms of acute gastroenteritis from November 2014 to June 2015. Samples were tested for Norovirus presence by RT-PCR, Rida quick Norovirus ICT antigen detection kit and IDEIA Norovirus EIA assay.

Fifty four out of 200 (27%) samples showed the 338-bp portion of the RdRp gene of both Norovirus genogroups I and II by RT-PCR (Figure 1).

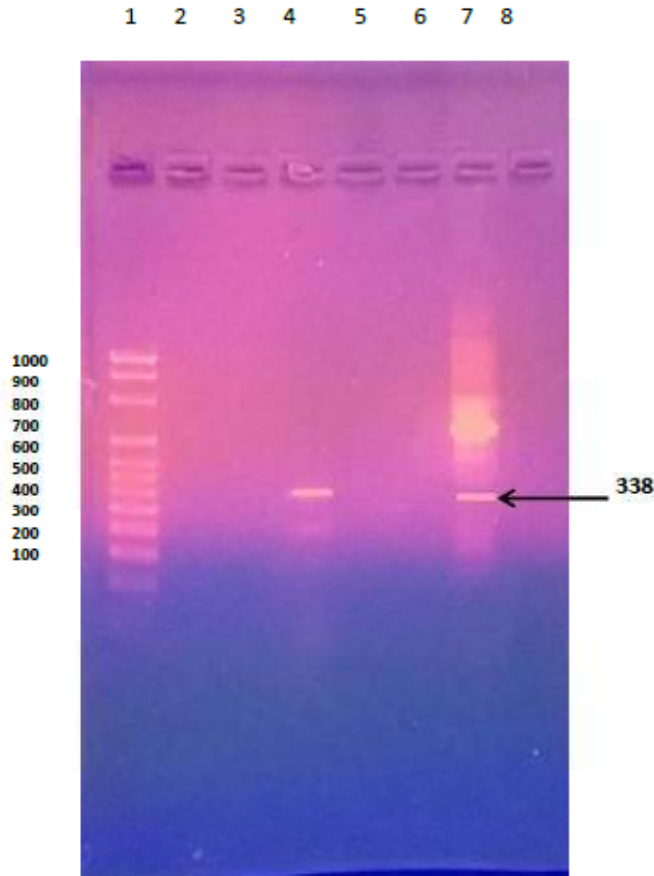


Figure 1: Lane 1: MW marker. Lanes 2,3,5,6,8: Negative samples. Lanes 4 and 7 positive samples with 338-bp portion of the *RdRp* gene of both Norovirus genogroups I and II by RT-PCR.

This study evaluated the diagnostic accuracy and analytical sensitivity of the latest version of the Rida quick Norovirus ICT antigen detection kit using RT-PCR as a reference method. Forty nine samples were positive by Rida quick kit, 46 of them were also

positive by RT-PCR. Whereas 8 of the 151 negative Rida quick samples were positive by RT-PCR. The assay showed high specificity (97.94%) and PPV (93.87%) and high sensitivity (85.18%) and NPV (94.7%) (table1).

TABLE (1): PERFORMANCE CHARACTERISTICS OF RIDA QUICK NOROVIRUS ICT ANTIGEN DETECTION KIT COMPARED TO RT-PCR

RT-PCR	RIDA QUICK NOROVIRUS ICT		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	46	8	54
NEGATIVE	3	143	146
TOTAL	49	151	200

DETECTION METHOD	ICT;	TIME TO COMPLETE TEST	15 MINUTES
SENSITIVITY	85.18 %	PPV	93.87%
SPECIFICITY	97.94 %	NPV	94.7%
AGREEMENT WITH PCR	49/54 (90.7%)	ACCURACY	94.5%

Table (2) shows the comparison between IDEIA Norovirus EIA and RT-PCR for identification of Norovirus. Forty four samples were positive by IDEIA Norovirus EIA, 35 of them were positive by RT-PCR. Whereas 19 of the 156 negative IDEIA

Norovirus EIA samples were positive by RT-PCR. The assay showed high specificity (93.8%) and PPV (79.55%) but low sensitivity (64.8%) with NPV (87.82%)

TABLE (2): PERFORMANCE CHARACTERISTICS OF IDEIA NOROVIRUS EIA ANTIGEN DETECTION KIT COMPARED TO RT-PCR

RT-PCR	IDEIA Norovirus EIA		Total
	Positive	Negative	
Positive	35	19	54
Negative	9	137	146
Total	44	156	200

Detection Method	EIA plate reader
Sensitivity	64.8%
Specificity	93.8%
Agreement with PCR	44/54 (81.48%)
Time to Complete Test	90 minutes
PPV	79.55%
NPV	87.82%
Accuracy	86%

DISCUSSION

Noroviruses are the major etiological agents of food borne disease as well as epidemic and sporadic cases of acute gastroenteritis all over the world. Therefore, quick laboratory diagnosis is an important tool to control NoV outbreaks by choosing the most suitable control practices such as improved cleaning and disinfection protocols, grouping, quarantine patients based on clinical data, exclusion of symptomatic food handlers or staff members, or, eventually, closing of hospitals' units(10). Significant improvement has been made in the diagnostic kits used for the detection of human noroviruses in the last few years. Most public health and research laboratories consider RT-PCR as the gold standard for norovirus detection. In areas where no laboratory facilities are available there is an increase need for sensitive, rapid and broadly reactive point-of-care assays, such as ICT assays, to allow simple, easy and trustworthynorovirus diagnosis. Molecular kits that can detect multi-gastrointestinal-pathogen in a single sample including norovirus are now available. These tests will be routinely done in many clinical laboratories over the next few of years (11).

The present work aimed to study the involvement of norovirus to sporadic cases of pediatric gastroenteritis in Zagazig University Hospitals and to assess the performance characteristics of the IDEIA norovirus assay (Oxoid, UK) together with a second assay, RIDA quick Norovirus Test (R-BioPharm, Darmstadt, Germany), for their capability to detect NoV antigen in faecal samples by comparing the obtained results with RT-PCR results.

In this work 54 out of 200 (27%) samples showed the 338-bp portion of the RdRp gene of both Norovirus genogroups I and II by RT-PCR. In a study done in Egypt by Zaghoul et al. (12) they detected norovirus in 16.2% of stool specimens from the children with gastroenteritis. Kamel et al. (13) studied the circulation of enteric viruses among the population of Cairo, Egypt, they documented that 26% of their samples were positive for norovirus. Lee et al. (14) studied the causative agents in 962 Korean children hospitalized with gastroenteritis they documented that norovirus was detected in 13.7% of the study population. Boga et al. (15) found that norovirus caused 8.6% of the positively diagnosed cases of sporadic pediatric gastroenteritis in Spain. Different detection methods, patients criteria, and time of

samples collection in these studies contribute to different results.

A critical parameter for evaluating the performance of ICT and EIAs is the selection of the reference method. This work used RT-PCR as the reference method to compare both Rida quick Norovirus ICT antigen RT-PCR. The assay showed high specificity (97.94%) and PPV (93.87%) and high sensitivity (85.18%) and NPV (94.7%) (Table 1).

Other investigators studied Rida quick Norovirus ICT antigen detection kit sensitivity and specificity compared to RT-PCR and documented variable results. Bruggink et al. (16) documented a sensitivity and a specificity of 87% and 97% correspondingly. The same authors documented a sensitivity of 83% and a specificity of 100% in a study conducted in 2011 (17). Bruins et al. (18) found a sensitivity of 57.1% and a specificity of 99.1%. Pombubpa and Kittigul (19) stated that the sensitivity and specificity of Rida quick are 83.3% and 87.5% respectively.

In this study 44 samples were positive by IDEIA Norovirus EIA, 35 of them were positive by RT-PCR. However 19 of the 156 negative IDEIA Norovirus EIA samples were positive by RT-PCR. The assay showed high specificity (93.8%) and PPV (79.55%) but low sensitivity (64.8%) with NPV (87.82%) (Table 2).

Gray et al. (20) determined test characteristics for IDEIA (Oxoid; UK). They found a sensitivity 58.93%, specificity 93.91%, PPV 92.30% and NPV 64.90%. Castriciano et al. (21) found that IDEIA has sensitivity and specificity of 60.6% and 100% respectively.

In a study done by Duizer et al. (22) they studied the IDEIA (Dakocytomation Ltd., Ely, UK) and they documented a sensitivity of 41% and specificity 98%. Khamrin et al. (23) evaluated the test characteristics of ELISA (Denka). They found a sensitivity 90.4%, specificity 96.4%, PPV 88.0%, NPV 97.2% and accuracy 95.0%. Costantini et al. (6) found that the sensitivity and specificity of the IDEIA Norovirus kit ranged from 57.4% to 77.8% and 79.7% to 91.9%, respectively, when the results were compared by different reference standards.

There are several reasons that could explain the variable sensitivity results. First, collection of samples after onset of symptoms by three days may play a role in lack of detection of NoV (7). Furthermore, long periods of fecal storage before testing may also affect the level of the virus, as proteolytic degradation may take place (24,25). Collection and examination of fecal samples within 72 h after onset of viral gastroenteritis symptoms was done in our study, while in other studies samples were tested after storage for 2 to 3

detection kit and IDEIA Norovirus EIA for diagnosing Norovirus.

Forty nine samples were positive by Rida quick kit, 46 of them were also positive by RT-PCR. Whereas 8 of the 151 negative Rida quick samples were positive by

years (26). Second, the outcome of all studies may be affected by differences in inclusion criteria fecal samples. Some studies included specimens only when the clinical symptoms indicated a NoV outbreak. On the other hand some studies tested all samples that were sent to the laboratory (24,25). Finally, the discrepancy in sensitivity of Rida quick and EIA kit found in different studies may be explained by the antigenic diversity of NoV. Certain genotypes may be missed because these kits use monoclonal and polyclonal antibodies formed against virus-like particles representing GI and GII genotypes.

The variable sensitivity of the assays raises serious questions about the usefulness of these kits for routine screening for NoV.

Previous studies have also encountered similar false-positive results with this EIA method (27). In this work, the nine samples that were negative by RT-PCR, but positive by EIA, also exhibited high absorbance values. The possibility of false negative RT-PCR results should be considered, this may be due to factors that might affect the sensitivity of the RT-PCR assay, e.g., the presence of PCR inhibitors in faecal samples, the instability of viral RNA and the viral genetic diversity (25).

In countries where the RT-PCR method has not been established for routine diagnosis, ICT RIDA quick Norovirus assay may be helpful for fast screening of norovirus infections in patients with acute gastroenteritis. In contrast, EIA assays investigated in this study was not satisfactorily sensitive to be effective as an exclusive diagnostic method for sporadic cases of NoV-associated acute gastroenteritis, but both tests cannot replace the RT-PCR method. Routine diagnosis of NoV diarrhoea may be inadequate due to the lack of a simple reliable test, and many laboratories do not have the facilities to conduct RT-PCR. Increasing the sensitivity of EIA method must be considered to become useful as a tool for the diagnosis of NoV diarrhoea in clinical laboratories.

Conclusion

The increasing rates of detection of NoV in pediatric patients suffering from acute gastroenteritis support the inclusion of NoV tests in screening of these patients together with other routine viruses tested.

The Rida quick and IDEIA Norovirus kits may be practical for rapid screening of fecal samples from patients with acute gastroenteritis.

Negative samples by these screening kits should be confirmed by a more sensitive and specific technique, such as RT-PCR.

REFERENCES

1. **Tsugawa T, Numata-Kinoshita K, Honma S, Nakata S, Tatsumi M, Sakai Y, et al.** Virological, serological, and clinical features of an outbreak of acute gastroenteritis due to recombinant genogroup II Norovirus in an infant home. *J. Clin. Microbiol.*,2006;177-182.<http://jcm.asm.org/content/44/1/177.full>10.1128/JCM.44.1.177-182.2006
2. **Payne DC, Vinjé J, Szilagyi PG, Edwards KM, Staat MA, Weinberg GA, et al.** Norovirus and medically attended gastroenteritis in U.S. children. *N Engl. J. Med.*, 2013;368:1121-1130.<http://www.ncbi.nlm.nih.gov/pubmed/23514289>10.1056/NEJMsa1206589.
3. **Hall AJ, Wikswo ME, Manikonda K, Roberts VA, Yoder JS, Gould LH.** Acute gastroenteritis surveillance through the National Outbreak Reporting System, United States. *Emerg. Infect. Dis.*, 2013;19:1305-1309.<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3739540/>[10.3201/eid1908.130482](https://doi.org/10.3201/eid1908.130482)
4. **Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, Green K, et al.** Proposal for a unified norovirus nomenclature and genotyping. *Arch. Virol.*, 2013; 158:2059-2068.<http://www.ncbi.nlm.nih.gov/pubmed/23615870>10.1007/s00705-013-1708-5
5. **Harris JP, Lopman BA, O'Brien SJ.** Infection control measures for norovirus: a systematic review of outbreaks in semi-enclosed settings. *J. Hosp. Infect.*, 2010;74:1-9.<http://www.ncbi.nlm.nih.gov/pubmed/19819586>10.1016/j.jhin.2009.07.025.
6. **Costantini V, Grenz L, Fritzing A, Lewis D, Biggs C.** Diagnostic accuracy and analytical sensitivity of IDEIA Norovirus assay for routine screening of human Norovirus. *Antonie van Leeuwenhoek*, 2010;74(8): 2770-2778.<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2916579/>[10.1128/JCM.00654-10](https://doi.org/10.1128/JCM.00654-10)
7. **Glass RI, Parashar UD, Estes MK.** Norovirus gastroenteritis. *N. Engl. J. Med.*, 2009;361:1776-1785.<http://www.ncbi.nlm.nih.gov/pubmed/19864676> PMID:19864676
8. **Ike A, Brockmann SO, Hartelt K, Marschang RE, Contzen M, Oehme RM.** Molecular epidemiology of Norovirus in outbreaks of gastroenteritis in Southwest Germany from 2001 to 2004. *J. Clin. Microbiol.*,2006; 44(4): 1262-1267.<http://www.ncbi.nlm.nih.gov/pubmed/16597849> PMID:16597849
9. **Schreier E, Doering F, Kunkel U.** Molecular epidemiology of outbreaks of gastroenteritis associated with small round structured viruses in Germany in 1997/98. *Arch. Virol.*, ;145:1-11.<http://www.ncbi.nlm.nih.gov/pubmed/10795514> PMID:10795514
10. **Barclay L, Park GW, Vega E, Hall A, Parashar U, Vinjé J, Lopman B.** Infection control for norovirus. *Clin. Microbiol. Infect.*,2014;20:731-740.<http://onlinelibrary.wiley.com/enhanced/doi/10.1111/1469-0691.12674/> 10.1111/1469-0691.12674
11. **Vinjé J.** Advances in laboratory methods for detection and typing of Norovirus. *J. Clin. Microbiol.*,2015;53(2):373-381.<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4298492/>[10.1128/JCM.01535-14](https://doi.org/10.1128/JCM.01535-14)
12. **Zaghloul MZ, El-Sahn SF, Galal ZA.** Coinfection of Rotavirus group A, Norovirus and Adenovirus in Egyptian children with gastroenteritis. *Life Science Journal*.2013; 10(2): 848-852.http://www.lifesciencesite.com/ljsj/life1002/119_18009life1002_848_852.pdfISSN:1097-8135
13. **Kamel AH, Ali MA, El-Nady HG, Rougemont A, Pothier P, Belliot G.** Predominance and circulation of enteric viruses in the region of greater Cairo, Egypt. *J. Clin. Microbiol.*,2009;1037-1045.<http://jcm.asm.org/content/47/4/1037.full>10.1128/JCM.01381-08
14. **Lee JI, Chung JY and Han TH.** Detection of human bocavirus in children hospitalized because of acute gastroenteritis. *J. Infect. Dis.*, 2007;196:994-997.<https://jid.oxfordjournals.org/content/196/7/994.full>10.1086/521366
15. **Boga JA, Santiago Melo A, Ine A, Nicieza, Isabel de Diego, Mercedes Villar, Francisco Parra, et al.** Etiology of sporadic cases of pediatric acute gastroenteritis in Asturias, Spain, and genotyping and characterization of Norovirus strains involved. *J. Clin. Microbiol.*,2004;42(6): 2668-2674.

- <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC427848/10.1128/JCM.42.6.2668-2674.2004>
16. **Bruggink LD, Dunbar NL, Marshall JA** Evaluation of the updated RIDA®QUICK (Version N1402) immunochromatographic assay for the detection of norovirus in clinical specimens. *J. Virol. Methods*,2015; 223:82-87.
<http://www.ncbi.nlm.nih.gov/pubmed/26248054> 10.1016/j.jviromet.2015.07.019
 17. **Bruggink LD, Witlox KJ, Sameer R, Catton MG, Marshall JA** Evaluation of the RIDA(®)QUICK immunochromatographic norovirus detection assay using specimens from Australian gastroenteritis incidents. *J. Virol. Methods.*, 2011;173(1):121-126.
<http://www.ncbi.nlm.nih.gov/pubmed/21295607>. 10.1016/j.jviromet.2011.01.017
 18. **Bruins MJ, Wolfhagen MJ, Schirm J, Ruijs GJ** Evaluation of a rapid immunochromatographic test for the detection of norovirus in stool samples. *Eur. J. Clin. Microbiol. Infect. Dis.*,2010;29(6):741-743.
<http://www.ncbi.nlm.nih.gov/pubmed/20306322>.10.1007/s10096-010-0911-5.
 19. **Pombubpa K, Kittigul L** Assessment of a rapid immunochromatographic test for the diagnosis of norovirus gastroenteritis. *Eur. J. Clin. Microbiol. Infect. Dis.*,2012; 31(9):2379-2383.
<http://www.ncbi.nlm.nih.gov/pubmed/22354525>10.1007/s10096-012-1579-9
 20. **Gray JJ, Kohli E, Ruggeri FM** .European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples. *Clin. Vaccine Immunol.* 2007; 14(10):1349-1355.
<http://www.ncbi.nlm.nih.gov/pubmed/17715333>PMID:17715333
 21. **Castriciano S, Luinstra K, Petrich A, Smieja M, Lee C, Jang D, Portillo E, et al.** Comparison of the RIDASCREEN norovirus enzyme immunoassay to IDEIA NLV GI/GII by testing stools also assayed by RT-PCR and electron microscopy. *J. Virol Methods.*, 2007;141(2):216-219.
<http://www.ncbi.nlm.nih.gov/pubmed/17208311>PMID:17208311
 22. **Duizer E, Pielat A, Vennema H, Kroneman A, Koopmans M** Probabilities in norovirus outbreak diagnosis. *J. Clin. Virol.*,2007; 40(1):38-42.
<http://www.ncbi.nlm.nih.gov/pubmed/17631044>PMID:17631044
 23. **Khamrin P, Nguyen TA, Phan TG, Satou k, Masuoka Y, Okitsu S, et al.** Evaluation of immunochromatography and commercial enzyme-linked immunosorbent assay for rapid detection of norovirus antigen in stool samples. *J. Virol. Methods*,2008;147(2):360-363.
<http://www.ncbi.nlm.nih.gov/pubmed/17959257>PMID:17959257
 24. **Dimitriadis A, Bruggink LD, Marshall JA.** Evaluation of the Dako IDEIA norovirus EIA assay for detection of norovirus using faecal specimens from Australian gastroenteritis outbreaks. *Pathology*, 2006; 38:157-165.
<http://www.ncbi.nlm.nih.gov/pubmed/16581657>PMID:16581657
 25. **Richards AF, Lopman B, Gunn A, Curry A, Ellis D, Cotterill H, et al.** Evaluation of a commercial ELISA for detecting Norwalk-like virus antigen in faeces. *J. Clin. Virol.* 2003; 26: 109-115.
<http://www.ncbi.nlm.nih.gov/pubmed/12589841>PMID:12589841
 26. **de Bruin E, Duizer E, Vennema H, Koopmans MP.** Diagnosis of norovirus outbreaks by commercial ELISA or RT-PCR. *J. Virol. Methods*, 2006;137:259-264.
<http://www.ncbi.nlm.nih.gov/pubmed/16901556>PMID:16901556
 27. **Schmid M, Oehme R, Schalasta G, Brockmann S, Kimmig P, Enders G** Fast detection using a real-time PCR assay and automated sample preparation. *BMC. Infect. Dis.*,2004; 4: 15.
<http://www.ncbi.nlm.nih.gov/pubmed/15186502>PMID:15186502