Egyptian Journal of Medical Microbiology Volume 29 / No.4 / October 2020 117-123 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Evaluation of Enzyme Immunoassay Method versus Real-time PCR for Diagnosis of *Rotavirus* Infection in Acute Infantile Diarrhea

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Key words:
Enzyme immunoassay; Real-time PCR; Rotavirus diagnosis

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ABSTRACT

Background*: Acute infectious gastroenteritis is a common cause of fatality between children in the developing countries which is usually due to viral etiology. Rotavirus is a ds-RNA (60-80nm), non-enveloped virus with a segmented genome. Group (A) of the virus is an important human pathogen that accounts for (90%) of the isolates. An easy, rapid, non-expensive and sensitive method is needed to detect this virus for clinical controlling.* ***The objective*** *of this study is to evaluate Enzyme immunoassay technique versus Quantitative real-time PCR in the diagnosis of infection with Rotavirus in the children with acute diarrhea.* ***Methodology****: This study was conducted on (75) infants and young children, from The Pediatric Department at Tanta University Hospitals in the period from December 2019 to March 2020 and were diagnosed according to history and clinical examination using Vesikari scoring system for acute severe gastroenteritis. Also, 10 healthy infants and children were taken as a control group. Stool samples were obtained from the patients and the controls. These specimens were tested with ELISA and Quantitative real-time PCR for detection of Rotavirus in stool.* ***Results****: The study revealed that 62 patients (82.6 %) were positive by ELISA and 74 cases (98.6 %) were positive with real time RT-PCR. Additionally, all the control group gave negative results by the two techniques.* ***Conclusion****: Enzyme immunoassay is an accurate and suitable method as a routine diagnostic measure for Rotavirus that can run a large number of samples. But, it is expensive when used for a single sample. Quantitative real time PCR was more sensitive and specific measure that can detect Rotavirus RNA in too minimal amounts in stools.*

INTRODUCTION

Acute infectious gastroenteritis is the most prevalent aetiology of death in children in the developing countries. It is usually due to viral infections[1,2]. The big hazard is commonly present in the young where fluid and electrolytes loss will lead to dehydration due to profuse vomiting and excessive diarrhoea. *Rotavirus* is a ds-RNA (60-80nm), non-enveloped virus with a segmented genome; six of these gene segments encode viral structural proteins (VP1 to VP4, VP6, and VP7), integrated into the virion while five segments encode for nonstructural proteins (NSP1, NSP2, NSP3, NSP4, and NSP5/NSP6). This virus has a characteristic wheel-like appearance by electron microscope (EM). Its name is derived from a latin word, meaning a wheel. Group (A) of the virus is an important human pathogen that accounts for 90% of the isolates[3]. In a body trial to avoid dehydration, the body extracts fluid from the intracellular space to conserve a constant blood volume, causing dehydration. So, it is advised to give rehydration solutions and adequate nutrients as early as possible according to the age[2]. *Rotavirus* can affect any age of human. Exposure to primary infection in the young is sometimes followed by severe and fatal diarrhea, while in the older, the clinical picture is often mild, mainly due to the development of immunity from recurrent multiple infections, but sometimes, severe disease can occur. The sequelae of infection are more severe in developing countries, where more than 500,000 deaths take place every year[4,5]. So, easy, quick and sensitive measure is critically needed to give fast diagnosis of *Rotavirus* for best clinical accurate control[6]. Other researchers, stated that, electron microscopy (EM) was the best method used for the recognition of *Rotavirus*. While, it is uncommonly used for its high expenses and requires skill necessities in addition to a decreased sensitivity. As a substitute for EM, Immunoglobulins against inner capsid viral protein (VP6) were used for diagnosis[6,7]. The most common biomarker for RVA infection is the highest copious viral protein. Several available tests including ELISA, agglutination of latex assays and immunochromatographic tests were used for the detection of RVA infection[8,9]. Now, the gold standard for RVAs diagnosis is the RT-PCR that is highly specific and sensitive molecular method. This molecular technique has increased the recognition frequency of

DOI : https://doi.org/10.51429/EJMM29415

Rotavirus in contrast with enzyme immunoassays [10,11]. This study aimed to evaluate Enzyme immunoassay technique versus Quantitative RT real-time PCR for diagnosis of *Rotavirus* infection in acute infantile diarrhea.

METHODOLOGY

Subjects

The study included (75) infants and young children with acute diarrhea from The Pediatric Department, Tanta University Hospitals from December 2019 to March 2020. Written consent was received from the mothers. Also, 10 healthy infants with no diarrhoeal history (3) weeks ago at least as controls.

Inclusion criteria: Infants and young children (6-60 months age) presented with acute diarrhea at the study time.

Exclusion criteria: The cases presented with blood in stool or those with persistent diarrhea since 3 weeks or more

Materials

- *Enzyme Immunoassay Test (ELISA):* (RIDASCREEN *Rotavirus*) (Biopharm AG- Germany- R).
- *RT real-time PCR for Rotavirus A in human stool:*
  - RNA Extraction kit (QIAamp Viral RNA Mini Kit) (QIAGEN®co).
  - *Rotavirus* Real Time PCR (VIASURE *Rotavirus* Real Time PCR Detection Kit. "CERTEST BIOTEC".

Methods:

Stool specimens were taken in sterile clean cups from (75) infants and young children with acute diarrhea (3 or more frequent loose faeces without blood during the day) and from the (10) control infants. Then they were sent to the Medical Microbiology and Immunology Dep., Tanta University as soon as possible in ice bags and were processed as follows

Enzyme Immunoassay Test (RIDASCREEN *Rotavirus*) (R-Biopharm AG, Germany):

A sandwiching type that utilizes monoclonal antibodies in a solid-phase EIA. These antibodies which are formed against (VP6), were coated to the inner surfaces of the wells in the provided microwell plate. The (VP6) is an antigen that is commonly present in all *Rotaviruses* causing human pathogenicity. Using a pipette to take the diluted stool suspension to be tested as well as the control samples into the well with monoclonal biotinylated anti-*Rotavirus* antibodies (Conjugate 1) to be incubated at room temperature (18-26°C), then wash followed by adding streptavidin poly-peroxidase conjugate (Conjugate 2) and incubated again at room temperature. If *Rotaviruses* were present, a complex was formed that is formed of fixed antibodies, the *Rotavirus* antigens and the antibodies conjugated with the biotin-streptavidin-peroxidase complex. Then wash again to remove the unbound (conjugate 2). Then add the substrate, the bound enzyme change the colour from colourless to blue if the reaction is +ve. Stop reagent caused color change from blue to yellow. The colour change was proportional to *Rotavirus* load.

Real-time PCR for *Rotavirus* in human stool:

Stool specimens were taken in clean sterile cups and examined as soon as possible. For delayed storage, the specimens were at first deeply frozen at (-20ºC). In our study, the specimen was completely thawed and carried to room temperature before work. Stool specimen was well homogenized. Stool samples were diluted perfectly before extraction. A pea-size stool (about 8mm) was taken and put in a 1.5 ml microcentrifuge tube that consists of (100 µl) of Phosphate Buffer Saline (PBS), intense vortexing and centrifuged (10,000 rpm) for 1min. (200 µl) of supernatant were used to perform RNA extraction.

RNA Extraction method:

The specimen was lysed in a high denaturation conditions to totally inactivate the RNases and to confirm intact viral RNA isolation. Buffers were adjusted to allow optimal RNA binding to the QIAamp membrane, then it was loaded on the QIAamp minispin column. Then, RNA was adsorbed on the silica membrane. Salt and pH adjusted that proteins and contaminants (enzymatic inhibitors) were completely removed from the membrane. The RNA bound to the silica membrane and contaminants were completely washed in two steps by 2 wash buffers that improved the eluted RNA purity. Pure RNA was produced in a special buffer (free of RNase) containing (0.04%) sodium azide to avoid growth of microbes. The purified eluted RNA is totally free from nucleases, contaminants or inhibitors. The advantage of the QIAamp membrane that it gives a high quality intact RNA in only twenty min. with no need for alcohol precipitation method nor phenol/chloroform extraction.

Real Time RT-PCR method:

The primers were chosen from the highly conserved region in group A *Rotavirus* non-structural protein 3 (NSP3) sequence as shown in table (1). The expected amplicon size was (87) bp. A fluorogenic probe was markedly labeled with FAM reporter at the 5'end and TAMRA quencher at 3'end.

Real Time PCR *Rotavirus* Detection Kit (VIASURE) has in every well all the essential constituents (buffer, specific primers /probes, polymerase dNTPS, reverse-transcriptase) in a stable form, also internal control to screen any inhibition of the reaction. The lyophilized *Rotavirus* positive control from red vial was prepared by addition of 100 µl water RNAse/DNAse free supplied and adequately vortexed.

Reconstitution of the wells: z"the number is decided" Rehydration buffer from the blue vial (15 µl) was equally added into every well.

Table 1: Nucleotide sequence and location of probe and primers in *Rotavirus* Non Structural Protein-3 (NSP3) region

Probe	Primers	Sequence of nucleotides (5'- 3')	Location
Probe		atgagcacaatagttaaaagctaacactgtcaa	984-1016
Rota (NVP3)-Forward		Accatctacacatgaccctc	963-982
Rota (NVP3)-Reverse		Ggtcacataacgcccc	1034-1049

Samples and controls:

RNA sample (5µl), prepared positive control from red vial or negative control from violet vial were put in different wells then closed with the caps. Centrifuged and loaded in thermocycler that was set up (Roche LightCycler ®96 Real-Time PCR System): Program will start the run.

Interpretation of the results:

Positive and negative controls are used in every run, gave the validation for the real time reaction by determining absence of the signal in negative control well and signal presence for positive control well. Internal control signal confirmed the correct performance of the amplification mix. The run was performed by the software Roche light cycler version 4.0.

Table 2: Sample interpretation; (+) amplification curve (-) No amplification curve

Interpretation of the results	Positive control well	Negative control well	Internal control well	*Rotavirus*
(Rotavirus A+ve)	+	-	+/-	+
(Rotavirus A -ve)	+	-	+	-
(Invalid)	+	+	+	+
(Invalid)	-	-	-	-

The PCR reaction inhibition was avoided by amplification of the internal control. Invalid result was registered if there was amplification signal in -ve control or absence of signals in the +ve control well. In case of invalid result, it was recommended to repeat the reaction again. The threshold cycle (Ct) value was detected by finding the point at which fluorescence exceeded a threshold limit of (0.04).

RESULTS

There was a clear significant difference inbetween the proposed three groups of age with obvious significant elevated number of patients in the two age groups (6-12m) and (13-30m) (P-value =0.002) as shown in table 3.

Table 3: Shows number of patients in relation to different age groups

Age (months)	Frequency	Percentage	Cum
6-12m	38	50.6%	50.6
13-30m	27	36.0%	86.6
31-60m	10	13.3%	100
X2	9.88		
P-value	0.002*		

Also, in relation to gender distribution, 51 males (68.0%) and 24 females (32.0%) were found with a high obvious significant difference (P-value =0.009) as shown in table 4.

Table 4: Distribution of Gender in patients' group

Gender	Frequency	Percentage	Cum
Male	51	68.0%	68
Female	24	32.0%	100
X2	6.48		
P-value	0.009*		

Also, (70.6%) of patients are from rural areas and (29.4 %) are from urban areas with a clear significant difference (p-value of <0.001). While 15 (20%) of patients with exclusive breast feeding and 50(66.6%) with bottle milk feeding and10 (13.3%) are weaned infants with a clear significant (P- value <0.001).

The majority of patients (42 %) were presenting with both vomiting and diarrhea followed by (36%) were presented with a fever, vomiting and diarrhea with an obvious significant (P-value <0.001).

Among both groups (N=85); 62 (72.9%) were +ve with RIDASCREEN ELISA while with VIASURE real time PCR detected 74 +ve cases for *Rotavirus A* infection (87.06%) .

While among the patients' group only; 62 (82.6%) +ve cases with RIDASCREEN and 74 (98.6%) +ve cases detected by real time PCR.

Table 5 showed that all control cases (N=10) were (-ve) for the virus with both tests.

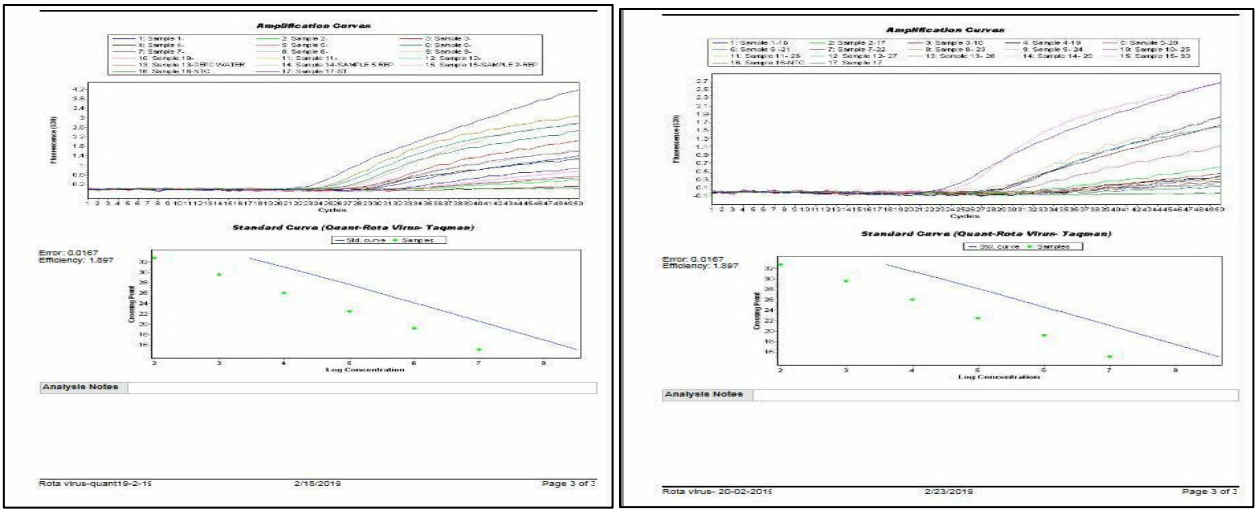

Fig. 1: Analysis of fecal samples by the VIASURE *Rotavirus* Real Time PCR Detection Kits

Table 5: Results obtained by both tests for the 2 studied groups (patients and controls).

	-ve	+ ve	Total
ELISA	23(27.05%)	62(72.9%)	85(100%)
PCR	11(12.95%)	74(87.05%)	85(100%)

Table 6: Results obtained by both tests in patients' group only

	-ve	+ve	Total
ELISA	13(17.3%)	62(82.6%)	75(100%)
PCR	1(1.3%)	74(98.6%)	75(100%)

Table 7: Diagnostic efficacy of ELISA test (RIDASCREEN) in comparison with the VIASURE real time PCR

True Positive	**62**
False Positive	**0**
True Negative	**1**
False Negative	**12**
Sensitivity	**83.7%**
Specificity	**100%**
PPV	**100%**
NPV	**11.11%**
Accuracy	**84%**

Table 8: Diagnostic accuracy of VIASURE real time PCR for *Rotavirus*.

Clinically diagnosed Patients	Real time PCR(VIASURE)		
	Negative	Positive	Total
Negative	10 (90.9%)	0 (0%)	10 (11.76%)
Positive	1(9.1%)	74(100%)	75(88.23%)
Total	11(100%)	74(100%)	85 (100%)

Table 9: Diagnostic efficacy of real time PCR in detection of *Rotavirus A* in stool samples.

	Sensitivity,	Specificity,	PPV,	NPV,	Accuracy,
Real time PCR	98.0%	100.0%	100.0%	90.9%	98.3%

DISCUSSION

The present work was conducted on (75) infants attending the Pediatric Department at Tanta University Hospitals from December 2019 to March 2020 and were clinically examined and using Vesikari scoring system for acute gastroenteritis grading. Additionally, ten healthy normal infants were participating as a control group. Stool specimens were taken from both groups.

Using the two diagnostic methods on the patients' group revealed that 62 cases (82.6%) were +ve by RIDASCREEN ELISA and 74 (98.6%) were +ve with VIASURE real time RT-PCR. While, the control group; all gave -ve results by 2 methods.

By ELISA test 62 patients (82.6%) were +ve and 13 patients (17.3%) were -ve for *Rotavirus* and when compared to real-time PCR; the sensitivity was (83.7%) and specificity was (100 %), PPV (100%), NPV (11.11%) and accuracy was (84 %).

In agreement with our ELISA results, Rashi Gautam, et al.[12] reported very close similar results using the RT-PCR as a gold standard measure. Also results provided by RIDASCREEN *Rotavirus* kit were (82.1%) sensitivity, (100%) specificity, (100%) PPV.

Similarly, Moutelíková et al. [13] detected close percentages with ours; the sensitivity and specificity for ELISA were (84.2% and 97.8%) respectively. Also, Sukran Artiran et al.[6] gave similar results by ELISA

with (94%) sensitivity, (100%) specificity and PPV = (100%). They preferred the use of commercial ELISA kits for screening large scales of samples as it is simple and easy to perform with high specificity and sensitivity in the diagnosis of *Rotavirus* in stool specimens**.**

On the contrary, Fruhwirth et al.[2] detected lower ELISA sensitivity with false +ve cases (12%) when compared with results of real time PCR while in our work ELISA gave no false positive results.

The ELISA sensitivity differs according to the time of stool sample taking related to the symptoms onset. Some samples contain a large amount of *Rotaviruses* that might cause "prozone" phenomenon that passively affects the ELISA results[14,15,16]**.** Also, ELISA sensitivity may decrease in the disease course as a result of immunostimulation against *Rotavirus*, producing mucosal antibodies which cover the virus and hindering its detection by ELISA [17]**.**

On the contrary to our ELISA results, Ojobor et al.[18] studied 146 acute gastroenteritis (AGE) cases and 33 healthy controls. Out of AGE subjects, (31.51%) were +ve to *Rotavirus* infection which is lesser than our percentage. None of the 33 controls were positive to RV infection that comes in agreement with our results**.** This difference may be due to that this area may contain other causes for (AGE).

Also on the contrary to our study, Amira et al.[31] detected only (20.8%) of the 120 stool samples that were positive for *Rotavirus*. This difference, which is a lesser percentage than ours, may be due to that they used the immunochromatographic technique which is less sensitive than ELISA that we used.

Regarding VIASURE *Rotavirus* Real Time PCR results; 74 patients (98.6%) gave positive results and one patient (1.3%) was negative. with 98% sensitivity, 100% specificity, 100% PPV, 90.9% NPV, 98.3% accuracy. Thus it is superior compared to ELISA.

In agreement with our results, Santiso-Bellon, et al.[10] reported that sensitivity was (97%) for detection of *Rotavirus* by real time PCR and it was higher than ELISA**.**

Similarly, Ye et al.[19] were in concordance with our results, found that real time PCR had very high sensitivity and specificity (99%) and (100%) respectively, while serological tests were less sensitive than real time PCR**.**

As a confirmation to our results, Yunjin Wang et al.[16] Liu et al.[8] and Corcoran et al.[20] declared that molecular assays like real time PCR are still the most sensitive while detection of antigen is just suitable for rapid diagnosis of *Rotavirus* infection and confirmed that real time PCR is the gold standard for diagnosis due to its high specificity and sensitivity. They also could get benefits about detection of minimal amounts of *Rotavirus* nucleic acids**.**

Also, Bennett et al.[21] andTate et al.[14] declared similar results and concluded that antibody related diagnostic methods are less sensitive by about (1000-10000) folds than RT- PCR**.**

Negative real time PCR results could be as the patient was not infected with *Rotavirus* or the virus may be so delicate to be destroyed during transport.

In our study, we found that 38 patients were between 6-12 months (50.6%), (27) patients (36%) were between 13-30 months and only (10) (13.3%) were between 31-60 months. There is an obvious significant increase in the age group more than 6 months and less than 30 months (p- value =0.002).

In agreement with our results, Joshua Gikonyo et al.,[22] declared that children aged from 13 to 24 months were with the highest incidence with *Rotavirus* infection (41%), while the least common were among the age group of (3 years and above)**.**

Supporting our results, Shaveta Dhiman et al.[9] found that the highest group of age was from 6 months to 24 months (85.71%)**.** Similar results were obtained by Surajudeen Junaid et al.,[3] Catherine Muendo et al.,[23] and El-Senousy, et al.[17] who declared that the highest age affected was (7-12 months) with ($P < 0.05$)**.**

The explanation for this *Rotavirus* infection distribution between different age groups refers to the early contact with contaminated surfaces as well as over-crowded communities [29,30].

In the present study, the ratio of participating males to females were 68% (51) to 32% (24) respectively, with a significant difference between both groups (P-value=0.009). All the males and 23 female patients gave positive results with real time PCR for *Rotavirus*. *Rotavirus* infection in males was significantly higher than in females.

In agreement with our results, Shaveta Dhiman et al.[9] declared that males were with the highest incidence of *Rotavirus* infection (90.5%) than females**.**

Also supporting our results, the study performed by Sally et al.,[15] who observed that males were highly affected by *Rotavirus* with 31 (62%) patients while females were 19 (38%) patients with statistically significant difference ($p>0.05$)**.**

Also the study of El-Senousy et al. [17] gave a similar results that males were more affected by *Rotavirus* than females**.**

The explanation for the higher incidence of male affection by *Rotavirus* may refer to the direction of parents to pay more attention to males than females as regard to hospital care[24,26,27]**.** Also, there is a proposition that female are more resistant to infections than males due to the presence of XX chromosomes that can alter tendency to different conditions[22,25,28.]

In our study, residence of (53) patients were from rural areas (70.6%) while only (22) patients (29.4%) were from urban areas. About (75 %) of cases with +ve *Rotavirus* diagnosed with real time PCR were of rural origin which gave a statistically significant increase in these cases.

In agreement with our study, Shaveta Dhiman et al.[9] stated higher incidence of *Rotavirus* infection were registered in rural areas**.** This could be explained by the very lower educational and socioeconomic levels with poor water supply that facilitate infection in these areas.

CONCLUSION

Enzyme immunoassay (ELISA) test is sensitive and specific in the diagnosis of *Rotavirus* infection. It is available for the routine diagnosis and easy in screening a very huge number of specimens. But, a main problem in ELISA is that it is too expensive if used to test single sample. Quantitative real time PCR has higher sensitivity and specificity. It also has the ability to detect *Rotavirus* nucleic acids in too minimal amounts in faecal specimens.

Recommendations

- Conduction of the study on a larger group with longer period to detect seasonal variations of *Rotavirus* and to start vaccination to all infants with risk factors.
- ELISA is suitable for the routine diagnosis and large number of specimens screening. While real time PCR should be used only for confirmation of diagnosis and the characterization of the *Rotavirus* strains.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Chukwu MO, Abia ALK, Ubomba-Jaswa E, Dewar JB, Obi C. Mixed Aetiology of Diarrhoea in Infants Attending Clinics in the North-West Province of South Africa: Potential for Sub-Optimal Treatment. Pathogens 2020; 9, 198.
2. Fruhwirth M, Brosl S, Ellemunter H, et al. Distribution of *Rotavirus* VP4 genotypes and VP7 serotypes among non-hospitalized and hospitalized patients with gastroenteritis and patients with nosocomially acquired gastroenteritis in Austria. J Clin Microbiol. 2000;38:1804-1806.
3. Surajudeen A Junaid, Chijioke Umeh, Atanda O Olabode et al. Incidence of *Rotavirus* infection in children with gastroenteritis attending Jos university teaching hospital, Nigeria. Virology Journal, 2011; 8:233.
4. Ai CE, Steele M, Lopman B. Disease burden and seasonal impact of improving *Rotavirus* vaccine coverage in the United States: A modeling study. PLoS One. 2020;15(2):e0228942. doi:10.1371/ journal.pone.0228942.
5. Hasson AJ. Prevalence of *Rotavirus* infection among children with acute gastroenteritis in thi-qar governorate. *Thi-Qar Medical Journal*. 2009; 3:88-100.
6. Sukran Artiran, Altay Atalay, Mehmet Adnan Ozturk et al. Investigation of *Rotavirus* with Various Methods in Children with Acute Gastroenteritis and Determination of Its Molecular Epidemiology in Kayseri Province, Turkey. Journal of Clinical Laboratory Analysis. 2017; 31: e22030.
7. Devdeep Mukherjee and Ritabrata Kundu: Laboratory diagnosis of *Rotavirus*. Pediatric infectious disease, 2014; 5:141-144.
8. Liu J, Kabir F, Manneh J, et al. Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhea: a multicenter study. Lancet Infect Dis. 2014; 14:716–724.
9. Shaveta Dhiman, Bimla Devi and Karnail Singh: Comparison of Enzyme-Linked Immunosorbent Assay and Immunochromatography for *Rotavirus* Detection in Children Below Five Years with Acute Gastroenteritis. Journal of Clinical and Diagnostic Research.2015; 9 (9):6-9.
10. Santiso-Bellon CS, Vila-Vicent, R. Falcon et al. Evaluation of VIASURE real-time PCR assays for detection of *Rotavirus* and *Norovirus* GI and GII in fecal samples. Journal of Clinical Virology. 2016; 82 S (S1–S142).
11. Stubbs SCB, Quaye O, Acquah M.E. et al. Full genomic characterization of a porcine *Rotavirus* strain detected in an asymptomatic piglet in Accra, Ghana. BMC Vet Res. 2020*;* 16, 11. https://doi.org/10.1186/s12917-019-2226-9.
12. Rashi Gautam, Freda Lyde, Mathew D. Esona et al. Comparison of Premier TM Rotaclone®, Pro Spec TTM, and RIDASCREEN® *Rotavirus* Enzyme Immunoassay Kits for Detection of *Rotavirus* Antigen in Stool Specimens. J Clin Virol. 2013; 58(1):292-294.
13. Moutelíková R, Dvořáková Heroldová M, Holá V, et al. Human *Rotavirus* A detection: Comparison of enzymatic immunoassay and rapid chromatographic test with two quantitative RT-PCR assays. Epidemiol. Mikrobiol. Imunol. 2019; 68(3):110–113.

14. Tate JE, Mijatovic-Rustempasic S, Tam KI et al. Comparison of 2 assays for diagnosing *Rotavirus* and evaluating vaccine effectiveness in chldren with gastroenteritis. Emerg. Infect. Dis. 2013; 19, 1245–1252.
15. Sally F. Lafta, Alaa H. Al-Charrakh, Abd-Alnabi J. Abd. Prevalence and Molecular Characterization Of *Rotavirus A* In Pediatric Patients With Acute Diarrhea. Euromediterranean Biomedical Journal. 2019; 14 (15) 65–69.
16. Yunjin Wang, Li Yu, Xu Zhou. Research progress for detection methods of *Rotavirus*. Hongkong Institute of Biologicals Standardization Limited. 2013; 2 (4), ISSN2305- 5154.
17. El-Senousy WM, Abu Senna ASM, Mohsen NA et al. Clinical and Environmental Surveillance of *Rotavirus* Common Genotypes Showed High Prevalence of Common P Genotypes in Egypt. Food Environ Virol. 2020; 12: 99–117. https://doi.org/10.1007/s12560-020-09426-0.
18. Ojobor, C.D., Olovo, C.V., Onah, L.O. *et al.* Prevalence and associated factors to *Rotavirus* infection in children less than 5 years in Enugu State, Nigeria. *VirusDis.* (2020). https://doi.org/10.1007/s13337-020-00614-x.
19. Ye S, Lambert SB, Grimwood K et al. Comparison of test specificities of commercial antigen-based assays and in-house PCR methods for detection of *Rotavirus* in stool specimens. J Clin Microbiol. 2015; 53, 295–297.
20. Corcoran MS, van Well, van Loo, et al. Diagnosis of viral gastroenteritis. in children: interpretation of real-time PCR results and relation to clinical symptoms. Eur J Clin Microbiol. Infect. Dis. 2014; 33, 1663–1673.
21. Bennett A, Bar-Zeev N, Jere KC et al. Determination of a viral load threshold to distinguish symptomatic versus asymptomatic rotavirus infection in a high-disease-burden African population. J. Clin. Microbiol. 2015; 53:1951– 1954.
22. Joshua Gikonyo, Betty Mbatia and Patrick Okanya et al. *Rotavirus* prevalence and seasonal distribution post vaccine introduction in Nairobi county Kenya; Pan African Medical Journal, 2019; 33:269.
23. Catherine Muendo, Ahmed Laving, Rashmi Kumar et al. Prevalence of *Rotavirus* infection among children with acute diarrhoea after rotavirus vaccine introduction in Kenya, a hospital cross-sectional study. BMC Pediatrics, 2018; 18: 323.
24. Soltan, MA, Tsai YL, Lee PA et al.: Comparison of electron microscopy, ELISA, real time RT-PCR and insulated isothermal RT-PCR for the detection of *Rotavirus* group A (RVA) in feces of different animal species. J Virol Methods. 2014: 235:99–104.
25. Rodak L, Valicek L, Smid B. et al.: An ELISA optimized for porcine epidemic diarrhea virus detection in feces. Vet. Microbiol. 2005; 1051: 9-17.
26. Florez ID, Niño-Serna LF, Beltrán-Arroyave CP. Acute Infectious Diarrhea and Gastroenteritis in Children. *Curr* Infect Dis Rep., 2020; 22(2):4. doi:10.1007/s11908-020-0713-6.
27. Gutierrez MB, Fialho AM, Maranhão AG, Malta FC, Andrade JSR, Assis RMS, Mouta SS, Miagostovich MP, Leite JPG, Machado Fumian T. *Rotavirus A* in Brazil: Molecular Epidemiology and Surveillance during 2018–2019. Pathogens. 2020; 9: 515.
28. Kim J, Kim HS, Kim et al.: Evaluation of an immunochromatographic assay for the rapid and simultaneous detection of *Rotavirus* and *Adenovirus* in stool samples. Ann. Lab. Med. 2014; 34:216–222.
29. De Graziaa S, Bonuraa F, Pepea A, Li Mulia S et al. Performance analysis of two immunochromatographic assays for the diagnosis of *Rotavirus* infection Journal of Virological Methods. 2017; 243: 50–54.
30. Slavica Mijatovic-Rustempasica, Mathew D. Esonaa, Alice L. Williams et al. Sensitive and specific nested PCR assay for detection of *Rotavirus A* in samples with a low viral load. J Virol Methods. 2016; 236: 41–46.
31. Amira E. Zaki, Wesam H. Amer, Ahmed A. Abo Elezz, and Waseela M. Mohamed. Study of Some Enteropathogens Causing Acute Diarrhea in Infants and Children Less than 5 Years Old. Egyptian Journal of Medical Microbiology, 2019; 28(2): 145-151. Online ISSN: 2537-0979.