Reduced Diagnostic Performance of Two Norovirus Antigen Enzyme Immunoassays for the Emergent Genogroup II Genotype 17 Kawasaki 2014 Variant

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Two commonly used norovirus enzyme immunoassays have reduced diagnostic performance, with clinical sensitivities ranging from 11% to 35% for the detection of the recently emerging genogroup II genotype 17 (GII.17) Kawasaki 2014 variant that caused the majority of infections in Asia during the winter of 2014 to 2015. False-negative results can compromise infection control and patient management.

Noroviruses are a major cause of acute gastroenteritis worldwide (1). Prompt and accurate laboratory confirmation of norovirus infections is important to identify the source and interrupt virus transmission. Norovirus antigen detection by enzyme immunoassays (EIAs) is commonly used in settings where molecular tests are not available. The performance of antigen-based EIAs is complicated, however, by the genetic diversity and evolution of noroviruses. Phylogenetically classified into at least 6 genogroups and subdivided into nearly 40 genotypes (2), noroviruses are genetically very diverse, and each genotype may represent a separate serotype (3). Furthermore, due to the low-fidelity RNA-dependent RNA polymerase, novel norovirus variants emerge periodically. For example, over the last 2 decades, new genogroup II genotypes 4 (GII.4) variants have emerged every 2 to 4 years (4, 5). In the winter of 2014 to 2015, a novel norovirus GII.17 Kawasaki 2014 variant replaced the predominant GII.4 Sydney 2012 variant in several Asian countries (6–9). Here, we report on the reduced diagnostic performance of two commonly used commercial norovirus EIAs for this emergent GII.17 variant.

A total of 90 stool samples that tested positive for norovirus by real-time reverse transcription-PCR (RT-PCR) were randomly selected from a pool of 141 samples for this study. They consisted of 25 GII.4 Sydney 2012-positive specimens and 65 GII.17 Kawasaki 2014-positive specimens. The samples were collected on presentation from patients that were hospitalized for norovirus gastroenteritis between December 2014 and March 2015, during which time the GII.17 Kawasaki 2014 variant outnumbered the GII.4 Sydney 2012 variant in Hong Kong (7). The median age of the patients was 19 years (interquartile range, 2 to 70 years). The female-to-male ratio was 1.4. Two norovirus EIAs were evaluated, Ridascreen norovirus 3rd generation antigen EIA (R-Biopharm) and IDEIA norovirus EIA (Oxoid), referred to as Ridascreen and IDEIA, respectively. The two kits were purchased in 2015 and were used before the indicated expiration dates. Testing was performed as per the manufacturers’ instructions by one experienced researcher who was blinded to the norovirus genotype information of the samples. Samples with readings in the equivocal range were counted as positive. All samples were kept frozen at −70°C before testing. Fecal norovirus RNA load was measured using a broadly reactive real-time RT-PCR assay and was expressed as a cycle threshold (C_T) value that was inversely proportional to the RNA copy number (10). The 95% confidence interval (95% CI) of clinical sensitivity was calculated using sampsize (available at http://sampsize.sourceforge.net). Spearman’s rank test was used to compare the correlations between C_T values and EIA optical density readings for each EIA norovirus genotype pair. Clinical and virologic factors (age, sex, norovirus genotype, and fecal norovirus load) that might be associated with the diagnostic performance of EIAs were analyzed using multivariate binary logistic regression. Likelihood was shown as the odds ratio (OR) with a 95% CI. Statistical analyses were performed using IBM SPSS Statistics version 22. A two-tailed P of <0.05 was considered statistically significant. Ethics approval for this study was obtained from the joint Chinese University of Hong Kong and New Territories East Cluster clinical research ethics committee (reference number CRE-2015.268).

Using real-time RT-PCR assay as the reference method, the clinical sensitivities of the two EIAs were significantly lower for GII.17 Kawasaki 2014 than for GII.4 Sydney 2012 (Ridascreen, 35% [95% CI, 24% to 48%] versus 80% [59% to 93%], P = 0.0002; IDEIA, 11% [4% to 21%] versus 56% [35% to 76%], P < 0.0001; Fisher’s exact test). Overall, optical density readings of the two EIAs correlated negatively and significantly with C_T values (Fig. 1). Notably, the strength of correlation was stronger for GII.4 Sydney 2012 (Spearman’s ρ = −0.88 and −0.81) than for GII.17 Kawasaki 2014 (Spearman’s ρ = −0.58 and −0.39) (Fig. 1). In addition, among the samples with high norovirus loads, defined as having a C_T value of less than an arbitrary cutoff of 20, false-negative results for the two EIAs were more commonly observed in GII.17 Kawasaki 2014 than in GII.4 Sydney 2012 (Ridascreen, 26% versus 6%, P = 0.2055; IDEIA, 70% versus 18%, P = 0.0016; Fisher’s exact test). Collectively, factors other than fecal norovirus...
load confounded the diagnostic performance of the two EIAs. To control the confounding effect, multivariate binary logistic regression analysis was performed. Norovirus GII.17 Kawasaki 2014 was independently associated with an increase in likelihood of obtaining a false-negative result for the two EIAs (Ridascreen, OR [95% CI], 11 [2 to 53]; IDEIA, 56 [4 to 1,000]) after adjustment for age, sex, and fecal norovirus load. Furthermore, in agreement with univariate analysis, a 1-unit increase in C_{R} value (i.e., a 2-fold decrease in norovirus load; fold change 2^{-ΔC{R}}) was independently associated with higher odds of obtaining a false-negative EIA result (Ridascreen, OR [95% CI], 1.6 [1.3 to 2.0]; IDEIA: 2.6 [1.5 to 4.3]). No association of diagnostic performance with the age and sex of patients was found.

With the simplicity of test procedures, norovirus antigen detection by EIAs is primarily used in laboratories without the sophisticated equipment used to perform more sensitive molecular norovirus RNA detection. In this study, we evaluated 2 commercially available EIAs, Ridascreen and IDEIA. Ridascreen has received clearance from the U.S. Food and Drug Administration for use in outbreak investigations and is required to test on at least 6 specimens to reach a clinical sensitivity of 90% or more (11, 12). Reduced diagnostic performance for the two EIAs (Ridascreen, OR [95% CI], 11 [2 to 53]; IDEIA, 56 [4 to 1,000]) after adjustment for age, sex, and fecal norovirus load. Furthermore, in agreement with univariate analysis, a 1-unit increase in C_{R} value (i.e., a 2-fold decrease in norovirus load; fold change 2^{-ΔC{R}}) was independently associated with higher odds of obtaining a false-negative EIA result (Ridascreen, OR [95% CI], 1.6 [1.3 to 2.0]; IDEIA: 2.6 [1.5 to 4.3]). No association of diagnostic performance with the age and sex of patients was found.

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In conclusion, our data demonstrate that two commonly used norovirus EIAs have reduced diagnostic performance for the emergent norovirus GII.17 Kawasaki 2014 variant. The potential of false-negative test results should be acknowledged, as this may compromise infection control and patient management. Emergence of a novel norovirus variant necessitates the continuous validation and refinement of norovirus antigen detection assays.

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M.C.W.C. and P.K.S.C. conceived the study. M.C.W.C designed and supervised the study. K.K. and T.-N.H. performed experiments. M.C.W.C. analyzed data and drafted the manuscript. All authors critically reviewed the manuscript with intellectual input and approved the final version. M.C.W.C. has access to all data and is responsible for the scientific integrity of this study.

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