Journal of Virological Methods 243 (2017) 50–54

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Performance analysis of two immunochromatographic assays for the diagnosis of rotavirus infection

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Article history:
Received 21 November 2016
Received in revised form 23 January 2017
Accepted 29 January 2017
Available online 31 January 2017

Keywords:
Rotavirus
Immunochromatography
Sentitivity
Specificity

A B S T R A C T

Group A rotaviruses (RVAs) are the primary cause of acute gastroenteritis (AGE) in young children worldwide. Several commercial tests including latex agglutination, enzyme-linked assays (ELISA) and immunochromatographic tests (ICT) have been developed for the diagnosis of RVA infection.

In the present study, the performance of two commercially available one-step chromatographic immunoassays, CerTest Rotavirus + Adenovirus (Biotec S.L, Zaragoza, Spain) and Vikia Rota-Adeno (bioMerieux SA, Lyon, France) were retrospectively evaluated using Real-time PCR as reference test. Re-testing by Real-time PCR of 2096 stool samples of children hospitalized with AGE previously screened by ICTs (1467 by CerTest and 629 by Vikia) allowed to calculate higher sensitivity for Vikia (94% vs 85% of CerTest) and higher specificity for CerTest (93% vs 89% of Vikia). Accordingly, higher Positive Predictive Values (87% vs 78%) and Positive Likelihood Ratios (12.32 vs 8.8) were found for CerTest and lower Negative Predictive Values (91% vs 97%) and Negative Likelihood Ratios (0.16 vs 0.06) for Vikia. However, both CerTest and Vikia showed a substantial agreement ($\kappa = 0.79$) with the Real-time PCR. A correlation between false negative results by ICTs and high Cycle Threshold values of Real-time PCR, indicative of low viral load, was observed. False positive results by the two ICT assays were not related to Norovirus, Adenovirus or Astrovirus infections, therefore the risk of cross-reactions was excluded. Both CerTest and VIKIA were able to detect the wide range of RVA genotypes circulating over the study period (including G1P[8], G2P[4], G3, G4, G9 and G12P[8]). The results of the present study showed a satisfactory efficacy of the two diagnostic tests analyzed.

1. Introduction

In industrialized countries, acute gastroenteritis (AGE) is a major cause of morbidity in infants and young children and constitutes a substantial burden in terms of medical and indirect costs. Group A Rotavirus (RVA), Adenovirus (AdV) type 40 and 41, Norovirus (NoV) and Astrovirus (AstV) are major gastroenteritis agents in infants and young children (Oude Munnink and van der Hoek, 2016; Simpson et al., 2003; Wilhelmi et al., 2003). RVAs are the primary cause of AGE in young children, with a death toll of 453,000, mainly in developing countries (Tate et al., 2012). Of the many RVA antigenic/genetic G/P combinations six genotypes are considered to be responsible for the vast majority of human cases in European countries: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] (Desselberger, 2014; Estes and Greenber, 2013). Accurate detection of RVA is essential for prevention and control of RVA disease and outbreaks monitoring. Historically, electron microscopy (EM) was first used for the detection of viral particles in stool samples (Soltan et al., 2014). However, EM observation is infrequently used as a routine diagnostic tool due to its high costs and the expertise requirements, joined to a reduced sensitivity. Starting from the 80s, diagnostic methods using monoclonal or polyclonal antibodies against the inner capsid protein VP6 were commercialized, replacing the EM test (Desselberger, 2014). The detection of VP6 protein in stools is generally used as a marker of RVA infection, being the most abundant viral protein, antigenically dominant and highly conserved among RVAs of different animal species. Several commercial tests including latex agglutination, enzyme-linked assays (ELISA) and immunochromatographic tests (ICT) were developed for the diagnosis of RVA infection. The ICTs are simple and rapid, provid-

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http://dx.doi.org/10.1016/j.jviromet.2017.01.025
0166-0934/

Table 1
Comparison of rotavirus detection results by CerTest and Vikia test related to Real-time PCR.

Test	No. of Samples					Sensitivity (95%CI)	Specificity (95%CI)	PPV (95% CI)	NPV (95% CI)	PLR	NLR
	Total	Positive by PCR and ICT (%)	Positive by ICT only (%)	Negative by PCR and ICT (%)	Negative by ICT only only (%)						
CerTest	1467	462 (31.49)	64 (4.36)	861 (58.69)	80 (5.45)	85% (80–90)	93% (90–95)	87% (83–92)	91% (88–94)	12.32	0.16
Vikia	629	173 (27.50)	48 (7.63)	398 (63.28)	10 (1.59)	94% (90–97)	89% (86–92)	78% (72–83)	97% (95–99)	8.8	0.06

ICT: ImmunoChromatographic Tests; PPV: Positive Predictive Values; NPV: Negative Predictive Values; PLR: Positive Likelihood Ratios; NLR: Negative Likelihood Ratios; CI: 95% confidence interval.

ing results in less than 30 min, making them an attractive diagnostic tool (Kim et al., 2014). Molecular techniques, such as reverse transcriptase polymerase chain reaction (RT-PCR) and Real-time PCR, are highly sensitive and specific, representing the gold standard for detection, genetic characterization and epidemiological studies of RVAs. However, not all diagnostic laboratory are equipped to carry out molecular investigations that require expensive equipment and advanced technical skills.

The aim of this study was to evaluate two commercial ICTs, CerTest Rotavirus + Adenovirus (Biotec S.L, Zaragoza, Spain) and Vikia Rota-Adeno (bioMerieux SA, Lyon, France) and compare their performance with the results of Reverse Transcription and Real-time PCR, used as the reference method. The sensitivity and specificity of the two tests used evaluated and related to the fecal viral load and the genotype of the RV strains. The risk of cross reactivity events due to AdV, NoV or AstV infection was also considered.

2. Materials and methods

In this study we performed a retrospective analysis of a total of 2096 stool samples, collected from children up to five years of age hospitalized for AGE from 2009 to 2015 at the "G. Di Cristina" Children Hospital of Palermo, Italy. All samples were screened for RVA infection at the diagnostic laboratory of ARNAS Civico Hospital, Palermo, using two different ICTs. In particular, 1467 samples, collected from January 2009 to April 2013, were tested with CerTest Rotavirus + Adenovirus (Biotec S.L, Zaragoza, Spain) and 629 samples, collected from May 2013 to December 2015, were screened with VIKIA Rota-Adeno (bioMerieux SA, Lyon, France). The ICTs were performed according to manufacturer instructions. Stool samples were stored at −20 °C until the biomolecular testing. All the samples collected, independently of the result of the screening test, were submitted to RNA extraction (QIAamp Viral RNA Mini Kit, Qiagen) and analyzed by random primers reverse transcription (Iturriza-Gomara et al., 1999) and Real-time PCR with specific primers targeting on the NSP3 gene of RVA (Pang et al., 2004). A Cycle thresholds (Ct) value ≤30 was defined as the cutoff to discriminate RVA positive samples. The RVA positive samples were G/P genotyped by RT-PCR of VP7 and VP4 genes followed by a hemi-nested multiplex PCR reaction using a mixture of specific primers for each genotype (genotypes G1-G4, G6, G8, and G9-G12 and P[4], P[6], P[8]-P[11] and P[14], for VP7 and VP4 types respectively) (Gentsch et al., 1992; Gouvea et al., 1990; Iturriza-Gomara et al., 2004). To evaluate infections by other enteric viruses, stool samples were also analyzed for the presence of NoV and AstV genome by in house PCR (Noel et al., 1995; Vinjé and Koopmans, 1996), while AdV antigens were searched by the same ICT assays used for the RVA screening (CerTest Rotavirus + Adenovirus and VIKIA Rota-Adeno).

2.1. Statistical analysis

For each ICT test (CerTest Rotavirus + Adenovirus – Vikia Rota-Adeno), the sensitivity, specificity, accuracy, predictive value, likelihood ratios, together with the confidence interval (CI) at 95%, were analyzed with respect to the reference Real-time PCR test. The Cohen's Kappa coefficient (κ) was used as a measure of the agreement between each ICT screened and the gold standard test (Real-time PCR). Agreement was graded as poor (0.0–0.20), moderate (0.20–0.40), fair (0.40–0.60), good (0.60–0.80), or substantial (0.80–1.00) (Viera and Garrett, 2005). The ability of each ICT to detect a range of RV genotypes was determined by calculating the percentage of strains of each genotype detected and the 95% CI. Significant differences were confirmed by calculating P values using the chi-square test.