Diagnostic Accuracy of Seven Commercial Assays for Rapid Detection of Group A Rotavirus Antigens

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Seven commercial immunochromatographic assays intended for the detection of group A rotavirus antigens in human stool samples were evaluated. These assays showed similar levels of diagnostic accuracy and were suitable for the detection of rotavirus in patients with acute gastroenteritis but missed some asymptomatic rotavirus shedding identified by real-time reverse transcription-PCR.

Group A rotaviruses (RVA) are a leading cause of severe gastroenteritis in young children worldwide, with an estimated 450,000 deaths of children under 5 years of age per year (1). Immunochromatographic (ICT) assays are widely used to detect RVA antigens in stool samples. A recent Australian study (2) demonstrated unexpectedly low specificity of the Vikia Rota-Adeno ICT assay (bioMérieux, Marcy l'Etoile, France), while excellent specificity was reported for this assay in previous studies (3, 4). This observation underlines the importance of regular assessments of the diagnostic performance of ICT assays in order to accurately diagnose RVA infections. In this study, we compared the diagnostic accuracies of seven commercially available ICT assays intended for the detection of RVA antigens in human stool samples, including the Vikia Rota-Adeno ICT assay.

The diagnostic accuracy of ICT assays was first assessed on a collection of surplus material from raw fecal samples collected for RVA screening by three French hospital laboratories (Charleville-Mézières, Dijon, and Saint-Etienne) during the RVA epidemic peak from February through May 2014. Whatever the rotavirus screening result, only specimens with enough material for the seven ICT assays and the reference test were included. Of note, because the asymptomatic individuals were not excluded, this first collection included patients with or without acute gastroenteritis (AGE) symptoms. Specimens were immediately stored at -20°C . The ICT assays were simultaneously performed on thawed samples from August to October 2014 at the National Reference Centre for Enteric Viruses, University Hospital of Dijon, France (NRCev), according to the manufacturer's instructions. The results of the assays were read by two laboratory technicians (plus a third laboratory technician in cases of discrepancies) blinded to the results of the initial rotavirus screening. The reference test was performed on the same thawed samples and on the same day as the ICT assays. This was an in-house real-time reverse transcription-quantitative PCR (RT-qPCR) assay adapted from the literature (5) and allowed the simultaneous detection of the RVA VP2 coding gene and pAW109 inhibition control RNA (Applied Biosystems, Foster City, CA, USA). The rotavirus strains were G and P genotyped according to the EuroRotaNet methods (www.eurorota.net/docs.php). For each ICT assay, the sensitivity, specificity, likelihood ratios, and diagnostic odds ratio were calculated using Meta-DiSc software (6) and the statistical analysis (Kruskal-

Wallis nonparametric test) was performed with Stata software (StataCorp release 12, College Station, TX, USA).

A total of 256 samples were included in the first collection. The ages of the patients ranged from 6 days to 72 years (median, 16.9 months), and the male/female ratio was 1.33. After exclusion of three samples because of an invalid result with the reference test (presence of RT-qPCR inhibitors), 110/253 (43.5%) samples were positive for RVA with the reference test. The 95% confidence intervals (95% CI) for each performance indicator overlapped, as summarized in Table 1, leading to the conclusion that the diagnostic accuracies of the seven ICT assays were similar. Overall, the ICT assays showed relatively weak sensitivity compared with RT-qPCR (from 69.1% to 78.2%), while the specificity of these assays was excellent ($\geq 97.9\%$). Interestingly, the specificity of the Vikia Rota-Adeno assay was 100%. In our study, the evaluation was done after optimization of the manufacturing process for Vikia Rota-Adeno (bioMérieux personal communication), which may explain the difference between the Australian study (2) and this study.

The positive likelihood ratio of the ICT assays was high (from 36.8 to 224.4), and the negative likelihood ratio was low (from 0.221 to 0.312), thus allowing high diagnostic odds ratio values ranging from 158.7 to 1,013.3. These data indicated a strong association between the results of the rotavirus ICT assays and the presence of the virus; these assays were therefore highly discriminatory (7, 8). RVA genome quantification revealed that the ability of the ICT assays to detect RVA was directly linked to the viral load, since the false-negative samples with ICT had a significantly lower viral load than the true-positive samples (Table 2). The sam-

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TABLE 1 Diagnostic accuracy of the seven rotavirus ICT assays calculated using 253 stool samples from the first collection^a

Assay (supplier; identifier)	% sensitivity (95% CI)	% specificity (95% CI)	Positive-likelihood ratio (95% CI)	Negative-likelihood ratio (95% CI)	Diagnostic odds ratio (95% CI)
Immunoquick NoRotAdeno (Biosynex; reference 0567_K10)	78.2 (69.3–85.5)	100 (97.5–100)	224.4 (14.1–3,577.2)	0.221 (0.156–0.314)	1,013.3 (60.8–16,875.1)
Vikia Rota-Adeno (bioMérieux; reference 31111)	77.3 (68.3–84.7)	100 (97.5–100)	221.8 (13.9–3,536.2)	0.231 (0.164–0.324)	962.3 (57.8–16,009.8)
Ridaquick Rotavirus/Adenovirus Combi (R-Biopharm; reference N1003)	76.4 (67.3–83.9)	100 (97.5–100)	219.2 (13.8–3,495.2)	0.240 (0.172–0.334)	915.2 (55.1–15,211.6)
Combi K-SeT Rota/Adeno (Coris BioConcept; reference K-1504)	75.5 (66.3–83.2)	100 (97.5–100)	216.7 (13.6–3,454.2)	0.249 (0.180–0.344)	871.4 (52.5–14,472.8)
Quick Rota/Adeno (ALL.DIAG; reference 5549)	69.1 (59.6–77.6)	100 (97.5–100)	198.5 (12.4–3,167.0)	0.312 (0.236–0.412)	636.4 (38.5–10,523.8)
Nadal Rota-Adenovirus test (Nal von minden; reference 481015)	73.6 (64.4–81.6)	99.3 (96.2–100)	105.3 (14.9–744.8)	0.265 (0.194–0.363)	396.6 (53.0–2,966.2)
SD Bioline Rotavirus (Standard Diagnostics, Inc.; reference 14FK10)	77.3 (68.3–84.7)	97.9 (94.0–99.6)	36.8 (12.0–113.4)	0.232 (0.164–0.328)	158.7 (46.5–541.5)
Pooled	75.3 (72.1–78.3)	99.6 (99.0–99.9)	85.1 (39.6–183.1)	0.253 (0.224–0.286)	368.9 (165.0–825.0)

^a Reference test, RT-qPCR. CI, confidence interval.

ples exhibiting a low viral load and leading to false-negative ICT results were typical of asymptomatic individuals. Indeed, among the 110 patients infected with RVA, the clinical presentations were successfully retrieved for 96 and revealed that only 79 (82.3%) of the patients presented AGE symptoms. Analysis of the RVA load according to the clinical presentation revealed that the viral loads were significantly higher ($P = 0.0003$) in individuals with AGE (median number of genome copies/g of feces, 1.23×10^{11} ; interquartile range, 1.08×10^{12}) than in asymptomatic individuals (median number of genome copies/g of feces, 1.35×10^5 ; interquartile range, 2.05×10^9). The sensitivity of the ICT assays was thus potentially higher in symptomatic individuals, with sensitivity ranging from 78.5% (95% CI, 67.8 to 86.9) to 88.6% (95% CI, 79.5 to 94.7) (data not shown), making these assays as efficient as the enzyme immunoassays recommended by the World Health Organization. Indeed, a study in symptomatic individuals revealed that the sensitivity of three commercially available enzyme immunoassays ranged from 75% to 82.1% compared to reverse transcription-PCR (9).

ICT assays were able to detect all the RVA genotypes present in the first collection, in which G9P[8] (34.5%) and G1P[8] strains (33.6%) predominated (Table 3). The ability of these assays to detect RVA strains bearing the most common genotypes not represented or underrepresented in the first collection was further assessed using a second collection of 15 RVA-positive stool samples, and the absence of cross-reactions was assessed using a third collection of 15 human stool samples that were negative for rotavirus but contained another enteric virus. These 30 additional samples were from the viral collections of the NRCev and were collected from patients with AGE symptoms. As shown in Table 3, all the RVA genotypes from the second collection were also detected by the ICT assays. Finally, and although assessed using a quite limited number of viruses, ICT assays did not cross-react with the other enteric viruses tested (Table 3).

In conclusion, the diagnostic accuracies of the seven ICT assays for RVA detection were similar, and these assays are suitable for the rapid diagnosis of RVA in individuals with AGE symptoms. However, the inability of the assays to identify asymptomatic in-

TABLE 2 Rotavirus load in true-positive and false-negative samples^a

Assay (supplier)	Rotavirus load										
	True-positive samples					False-negative samples					P value
	No.	Min.	Max.	Median	IQR	No.	Min.	Max.	Median	IQR	
Immunoquick NoRotAdeno (Biosynex)	86	6.37×10^5	1.92×10^{13}	2.96×10^{11}	1.18×10^{12}	24	0.24×10^3	6.09×10^7	3.78×10^3	2.38×10^5	0.0001
Vikia Rota-Adeno (bioMérieux)	85	6.37×10^5	1.92×10^{13}	3.27×10^{11}	1.18×10^{12}	25	0.24×10^3	6.09×10^7	3.82×10^3	2.42×10^5	0.0001
Ridaquick Rotavirus/Adenovirus Combi (R-Biopharm)	84	6.37×10^5	1.92×10^{13}	3.58×10^{11}	1.29×10^{12}	26	0.24×10^3	6.09×10^7	9.16×10^3	6.07×10^5	0.0001
Combi K-SeT Rota/Adeno (Coris BioConcept)	83	6.37×10^5	1.92×10^{13}	3.88×10^{11}	1.40×10^{12}	27	0.24×10^3	7.69×10^7	1.45×10^4	6.50×10^5	0.0001
Quick Rota/Adeno (ALL.DIAG)	76	6.37×10^5	1.92×10^{13}	4.28×10^{11}	1.42×10^{12}	34	0.24×10^3	3.76×10^{12}	1.85×10^5	1.13×10^7	0.0001
Nadal Rota-Adenovirus test (Nal von minden)	81	6.37×10^5	1.92×10^{13}	3.99×10^{11}	1.38×10^{12}	29	0.24×10^3	7.69×10^7	3.14×10^4	1.80×10^6	0.0001
SD Bioline Rotavirus (Standard Diagnostics, Inc.)	85	1.64×10^3	1.92×10^{13}	3.27×10^{11}	1.18×10^{12}	25	0.24×10^3	6.09×10^7	1.45×10^4	6.07×10^5	0.0001

^a $n = 110$ rotavirus-positive samples with the reference test. Data in the “No.” columns represent numbers of rotavirus samples. Data in the “Min.” (minimum), “Max.” (maximum), “Median,” and “IQR” (interquartile range) columns represent numbers of genome copies per gram of feces.

TABLE 3 Rotavirus results for each ICT assay according to RVA genotypes and other enteric viruses tested

Genotype(s) or virus	No. of strains	No. (%) of positive samples						
		Biosynex (Immunoquick NoRotAdeno)	bioMérieux (Vikia Rota-Adeno)	Standard Diagnostics (SD Bioline Rotavirus)	R-Biopharm (Ridaquick Rotavirus/ Adenovirus Combi)	Coris BioConcept (Combi K-SeT Rota/Adeno)	Nal von minden (Nadal Rota-Adenovirus test)	ALL.DIAG (Quick Rota/Adeno)
RVA genotypes (first collection)								
G9P[8]	38	36 (95)	35 (92)	34 (89)	34 (89)	34 (89)	34 (89)	33 (87)
G1P[8]	37	34 (92)	34 (92)	34 (92)	34 (92)	33 (89)	33 ^b (89)	30 (81)
G3P[8]	6	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
G3P[9]	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
G2P[4]	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
G3P[4]	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
G3 + G9P[8]	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
G1 + G9P[8]	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
G-UD ^a P[8]	3	2 (67)	2 (67)	2 (67)	2 (67)	2 (67)	0 (0)	0 (0)
G1P-UD ^a	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G-UD ^a P-UD ^a	18	1 (6)	1 (6)	2 (11)	1 (6)	1 (6)	1 (6)	1 (6)
Total	110	86 (78)	85 (77)	85 (77)	84 (76)	83 (75)	81 (74)	76 (69)
RVA genotypes (second collection)								
G2P[4]	7	6 (86)	6 (86)	6 (86)	7 (100)	6 (86)	6 (86)	7 (100)
G12P[8]	5	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
G4P[8]	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	1 (50)
G4P[4]	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total	15	14 (93)	14 (93)	14 (93)	15 (100)	14 (93)	13 (87)	14 (93)
Other enteric viruses (third collection)								
Norovirus ^c	8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sapovirus ^d	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Astrovirus ^e	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Adenovirus ^f	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aichi virus ^g	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	15	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

^a UD, undetermined.^b One sample with a discrepant result was identified as negative after a reading by a third laboratory technician. Reader agreement was 100% for all other samples and for all other ICT assays.^c The following norovirus genotypes were included: GI.2 (*n* = 1), GI.3 (*n* = 1), GI.4 (*n* = 1), GII.2 (*n* = 1), GII.4 (*n* = 3), and GII.6 (*n* = 1).^d The following sapovirus genotypes were included: G1.1 and G1.2.^e The strains were astrovirus type 2.^f The strains were adenovirus type 41.^g The strain was Aichi virus B.

dividuals could raise concerns about the prevention and control of RVA transmission, for example, in nosocomial infections. When it is necessary to detect low viral loads, RT-qPCR should be considered due to its better sensitivity.

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We declare that we have no conflict of interest to disclose.

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