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Short Communication

Evaluation of real-time RT-PCR Rhino&EV/Cc r-gene® (bioMérieux) kit versions 1 and 2 for rhinovirus detection

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ABSTRACT

Background: Human rhinoviruses (HRVs) are frequent etiologic agents of tract infections, ranging from benign upper to potentially life-threatening lower respiratory tract infections. Diagnosis is based on molecular methods. 169 HRV types, belonging to species A, B and C, have been identified. This high genetic diversity makes it difficult to accurately detect circulating HRVs and to diagnose severe infection.
Objectives: To comparatively assess the ability to detect HRV clinical isolates of the first version (V1) of the commercial real-time RT-PCR Rhino&EV/Cc r-gene® (bioMérieux) kit, of an in-house RT-PCR followed by genotyping, considered as the reference method, and of the second version of this commercial test (V2).
Study design: From September 2011 to April 2013, HRVs were prospectively detected in 2525 respiratory specimens, using V1 in combination with the in-house reference RT-PCR. In November 2013, 85 specimens that had given initially false negative results with V1 were retested simultaneously with V1 and V2 and the in-house RT-PCR. In addition, 421 negative specimens with the in-house assay were prospectively tested with V2.
Results: Among the 2525 specimens, V1 detected 80.7% (502/622) of in-house RT-PCR positive isolates: 85.3% (220/258) of HRV-A, 84.4% (27/32) of HRV-B and 74.9% (176/235) of HRV-C. Among the 85 respiratory samples tested with V1, V2 and the in-house RT-PCR, V2 was more efficient than V1 in detecting 16 HRV isolates: 11/33 (33.3%) of HRV-A and 5/47 (10.6%) of HRV-C tested. The analytical sensitivity of V2 was greater for 8/18 HRV-A genotypes and 2/22 HRV-C genotypes. Relative to the in-house assay, the specificity of V2 was 100% (421/421).
Conclusions: This study showed a slightly higher sensitivity of V2. However, diverse genotypes, especially HRV-C, were undetected.

1. Introduction

Human rhinoviruses (HRVs) are the most frequent agents of upper respiratory tract infection. They are also associated with exacerbation of chronic respiratory diseases and potentially life-threatening severe lower respiratory tract infections. Molecular methods of genomic detection are essential for the diagnosis of severe HRV infections. 169 HRV types have been identified and classified into three species A, B and C [1]. This high genetic diversity makes it difficult to accurately detect circulating HRVs and to diagnose severe infection using molecular assays.

2. Objectives

The aims of this study were to compare the efficacy of the first version (V1) of the commercial real-time RT-PCR Rhino&EV/Cc r-gene® (bioMérieux) kit in detecting HRV clinical isolates and that of an in-house-RT-PCR followed by genotyping, considered as the reference method, and to assess whether HRV detection was improved with the second version of this commercial test (V2).

3. Study design

Nasopharyngeal swabs, immediately placed in a tube with 3 mL universal viral transport medium, and nasopharyngeal aspirates

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Table 1
Results of HRV detection on 2525 respiratory samples with the in-house classic RT-PCR (reference method) and the real-time RT-PCR Rhino&EV/Cc r-gene® (bioMérieux) kit version 1 (V1).

V1	In house RT-PCR		
	Positive	Negative	Total
Positive	502	0	502
Negative	120	1903	2023
Total	622	1903	2525

were collected in patients with suspected HRV infection. Nucleic acids were extracted from 400 μl of sample with the NucliSENS® EasyMAG® automated system (bioMérieux), following the specific B protocol. Since 2009, prospective HRV detection has been performed at the teaching hospital of Clermont-Ferrand (France) with an in-house classic one-step RT-PCR targeting the hypervariable region in the 5′-UTR (untranslated region), and the 1A and 1B regions encoding the VP4 and VP2 capsid proteins (amplicon of 549 nt) [2]. This assay amplifies all three species of HRV as well as numerous genotypes of enterovirus (EV) (*unpublished data*). PCR products were visualized after gel electrophoresis and fluorescent staining (Gelstar, Lonza). We used an appropriate DNA ladder to control the size of PCR products and to estimate quantification. All positive HRV and EV samples are prospectively genotyped from these PCR products with a second round of the same primers, based on phylogenetic analysis of the 1A/1B genomic region [3]. From September 2011 to April 2013, the first version (V1) of the commercial real-time RT-PCR Rhino&EV/Cc r-gene® (bioMérieux) kit was used for prospective HRV testing in combination with the in-house RT-PCR. This commercial test is a duplex real-time RT-PCR that amplifies both HRV and EV genomes by targeting 5′UTR (amplicon of 157 nt for HRVs and 146 nt for EVs) without differential detection. Cellular control assesses the quality of the sample collection by validating the presence of cells and the absence of inhibitors.

In September 2013, the V2 kit was launched with the addition of novel primers expected to improve HRV detection. In November 2013, we tested V1 false negative samples (in-house RT-PCR positive, HRV confirmed by genotyping, V1 negative) to determine whether detection was improved with the V2 version. The samples were a representative panel of circulating isolates from the two previous years. Specimens were stored at −20 °C upon arrival and anonymized for the study. Immediately after extraction, the three tests were concomitantly performed. V1 and V2 assays were performed with the Rotor-Gene Q® (Qiagen) and interpreted according to the manufacturer's instructions. To have a more accurate comparison of the performances of the three assays, we classified PCR results according to a semi-quantitative estimation of viral load. In-house RT-PCR positive and low positive classification was obtained by comparison of RT-PCR products and DNA mass ladder band intensity on agarose gel electrophoresis. For V1 and V2 assays, samples that had Ct (cycle threshold) lower than 37.5 cycles were considered as positive, between 37.5 and 40 as low positive, and greater than or equal to 40 as negative. We considered that HRV detection with V2 was improved when V1 was negative and V2 was low positive or positive and when V1 was low positive and V2 positive. Any other combination of results was considered to indicate equal efficacy of the two assays. In addition, 421 negative specimens with the in-house assay were prospectively tested with V2.

4. Results

Of the 2525 respiratory samples tested for HRVs or EVs, 622 (24.6%) were positive with the in-house RT-PCR and 502 (19.9%) with V1 (Table 1). Of the 120 V1 false negative samples, 84 were classified as positive and 36 as low positive with the in-house assay. Overall percent agreement (OPA) of V1 *versus* the in-house RT-PCR was 95.2% (2405/2525). Positive percent agreement (PPA) of V1 *versus* the in-house RT-PCR was 80.7% (502/622). Genotyping of the 622 positive samples showed that V1 detected 85.3% (220/258) of HRV-A, 84.4% (27/32) of HRV-B, 74.9% (176/235) of HRV-C and 96.6% (28/29) of EVs. Owing to low viral load and/or EV/HRV co-infection, genotyping failed for 68 samples (68/622, 10.3%), of which 51 were positive with V1.

To assess the performance of the V2 kit, we tested 85 available samples among the previously identified 120 V1-false negative specimens. HRV genome was detected with the in-house RT-PCR in 82 samples (58 positive, 24 low positive) (Table 2). These isolates comprised 33 HRV-A (18 genotypes), 2 HRV-B (1 genotype) and 47 HRV-C (22 genotypes). Three samples initially selected as low positive with the in-house RT-PCR were negative with the three assays. V1 was negative for 75/82 HRV positive samples. Among those 75 V1 negative samples, 61 were negative with V2, five were low positive and nine were positive. V2 detected a total of 14 supplementary isolates: 9 HRV-A (1 A15, 1 A18, 2 A29/44, 1 A33, 1 A71, 1 A80/46, 2 A102) and 5 HRV-C (2 C8, 1 C16, 1 C38, 1 C_pat10). Seven specimens initially tested negative with V1 were re-classified as low positive with this same assay (median Ct: 38.7). Among those seven V1 low positive samples, two were negative with V2 (A12 and A101), three were low positive and two were positive (A20 and A29/44).

In all, V2 was negative for 63/82 specimens, low positive for 8 (median Ct: 38.8) and positive for 11 (median Ct: 33.6). According to the criteria of improved detection mentioned above, V2 was more efficient than V1 in detecting 16 HRV isolates: 11/33 HRV-A tested (33.3%) and 5/47 HRV-C tested (10.6%). Detection was improved for 8/18 HRV-A genotypes (A15, A18, A20, A29/44, A33, A71, A80/46, A102) and 2/22 HRV-C genotypes (C8, C38).

We tested 421 samples with V2 that were negative with the in-house RT-PCR. All were negative.

5. Discussion

We demonstrated that the PPA of the V1 assay *versus* the in-house RT-PCR (our reference test) was 80.7%, with detection performances ranging from 75% for HRV-C to 85% for HRV-A. To assess improvement in performance of the V2 assay, we selected 85 V1-false negative respiratory specimens. V1 provided low positive results for seven specimens (five HRV-A, two HRV-C) initially tested negative in prospective diagnosis. This lack of reproducibility probably reflects low viral load. Isolate detection was improved in V2 for 33.3% of HRV-A and 10.6% of HRV-C. We demonstrated enhanced analytical sensitivity of V2 for eight HRV-A genotypes (A15, A18, A20, A29/44, A33, A71, A80/46, A102) and two HRV-C genotypes (C8, C38). We assessed that the specificity of V2 was 100% (421/421). On the hypothesis that all isolates detected by V1 would be detected by V2 and since V2 detected 16/82 isolates tested, we estimated that the PPA of V2 was 83.3% (518/622) minimum and that the OPA of V2 *versus* in-house RT-PCR was 95.9% (2421/2525) minimum. Although V1 detected 85.3% of HRV-A infections, certain genotypes such as A12 and A78 remained undetected by V2. We tested two HRV-B42 isolates of species B. Neither was detected by V1 or V2 whereas V1 detected a sample containing HRV-B42 cultured strain in the 2012 Quality Control for Molecular Diagnostics (QCMD) program. This QCMD sample VP4/VP2 sequence (accession number KJ934989) is identical to the ATCC® strain VR-338 sequence (accession number FJ445130), which was collected in the early 1960s. This underlines the necessity of testing diagnostic methods with recent circulating isolates rather than with reference strains. Detection of HRV-C16 (1/3 isolates) and of HRV-C_pat10 (1/9 isolates) with V2 was unsatisfactory and the kit was still unable to detect 18 HRV-C genotypes tested (31