Usefulness of Published PCR Primers in Detecting Human Rhinovirus Infection

Faux, Cassandra; Arden, Katherine E; Lambert, Stephen B; Nissen, Michael D; Nolan, Terry; Chang, Anne; Sloots, Theo P; Mackay, Ian M

Published in:
Emerging Infectious Diseases

DOI:
10.3201/eid1702.101123

Published: 01/01/2011

Document Version
Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 17. Nov. 2019
Usefulness of Published PCR Primers in Detecting Human Rhinovirus Infection

Cassandra E. Faux, Katherine E. Arden, Stephen B. Lambert, Michael D. Nissen, Terry M. Nolan, Anne B. Chang, Theo P. Sloots, and Ian M. Mackay

We conducted a preliminary comparison of the relative sensitivity of a cross-section of published human rhinovirus (HRV)–specific PCR primer pairs, varying the oligonucleotides and annealing temperature. None of the pairs could detect all HRVs in 2 panels of genotyped clinical specimens; >1 PCR is required for accurate description of HRV epidemiology.

Human rhinoviruses (HRVs) cause more asthma exacerbations than any other known factor, in addition to causing most colds and influenza-like illnesses. The prevalence of HRV in published reports varies considerably. A novel HRV clade identified in 2006, now known as HRV species C (HRV-C) (1), can be identified only by PCR. Since 1988, seasonality and clinical outcomes and numerous different primer pairs have been used to identify HRV; how well these methods perform on new HRV types is uncertain. Given the likely variation in the preparation of RNA, the quality and formulations of commercial reverse transcription (RT)-PCR enzymes and reaction mix components and changes in thermal cyclers since 1988, not surprisingly many, perhaps most, of these assays are not being used in the manner they were originally described. For example, the first HRV-specific primers reported (2) have subsequently been used with different RNA preparation methods, amounts of reverse transcriptase, cDNA priming strategies, dNTP concentrations, annealing temperatures (Tₘ), and cycling conditions (3,4).

The Study

We conducted a preliminary comparison of the relative sensitivity of a cross-section of published HRV-specific PCR primer pairs (most of which were first published before HRV-C was reported), independent of most variables described above, by testing a panel of 57 clinical specimen nucleic acid extracts from combined nose and throat swabs from preschool children with colds and influenza-like illnesses in Melbourne, Australia. The study was approved by the Royal Children’s Hospital Human Research Ethics Committee. The panel included representatives of the 3 HRV species (Figure), human enteroviruses (HEVs), and extracts negative for picornaviruses. The HRVs had been previously detected by using a nested primer pair (online Appendix Table, www.cdc.gov/EID/content/17/2/294-appT.htm) (5). We used 10 different HRV primer pairs and also retested specimens by using the original primer pair with our standard reagents and equipment (5). We applied the published Tₘ when possible. The original descriptions of primer pairs 7 and 10 (online Appendix Table) lacked Tₘ information, and after in-house calculations, we used Tₘs of 50°C and 58°C, respectively. We also deliberately standardized the reagents (OneStep RT-PCR kit, QIAGEN, Doncaster, Victoria, Australia) and thermal cyclers used (Veriti, Applied Biosystems, Foster City, CA, USA) for conventional PCR and the RotorGene 3000 real-time cycler (QIAGEN). Because primer pair 1 had a published history of detecting types from all HRV species, we chose it to genotype HRV-positive samples by sequencing the amplified products. Other pairs were used if pair 1 was unsuccessful.

We found that no primer pair detected the same HRVs and HEVs typed when the original pair (5) or pair 1 (online Appendix Table) was used. Five primer pairs, including real-time PCR (rtPCR) pair 5, did not amplify the HEVs, a positive feature for HRV-specific studies. Only 2 primer pairs amplified anything from a specimen that was positive for both HRV and HEV, a problem for accurate estimation of the frequency of co-detections. The original primer pair screen detected 3 untypeable picornaviruses, which were not detected by any other pair or by repeat testing using the same pair. Only the second-round amplicon of the 3 nested sets of nested primer pairs (2, 3, and 9) was considered because the second round increased the total number of positive specimens over the first round. The longest amplicon, produced by primer pair 7, was also a valuable genotyping target, but it detected only 14 of the original 27 HRV-positive specimens in this population.

We next selected 4 frequently published primer pairs (1, 5, 7, and 8) to examine 44 picornavirus-positive specimens (39 HRVs, 3 HEVs, and 2 untypeable picornaviruses) from nonhospitalized children with acute asthma exacerbation (6). As before, primer pair 1 detected the greatest num-
PCR Primers and Detecting Rhinovirus

Let's compare the number of HRV- and HEV-positive specimens detected by other primer sets (n = 41), followed by pair 7 (n = 40), pair 5 (n = 36), and pair 8 (n = 31). Most notably, primer pair 7 performed better than it had in the previous population, detecting only 1 fewer HRV than primer pair 1 and 9 more HRVs than pair 8. No species-specific bias was apparent, but generally, a specimen with a lower RNA concentration, as indicated by the cycle threshold from primer pair 5, was less likely to be detected or typed by using other primer pairs. Primer pairs 5 and 8 did not detect the 3 HEVs (HEV-68).

Many possible reasons could cause discrepant virus testing results between different sites, including changes to specimen integrity resulting from transport and variable amplification resulting from low viral loads. The effects of viral load can be seen in this study: specimens in population 1 that were positive with multiple (>6 separate pairs) primer pairs had a mean cycle threshold of 33.3 (combining results from both rtPCRs), whereas those with <6 positive results had means of 39.3 cycles. Most (29/33) specimens with <3 positive primer pairs were negative by rtPCR. Amplification variability can also be attributed to the substantial nucleotide sequence diversity between HRVs and the different temporal and clinical characteristics of the 2 specimen populations we used. Population diversity is a feature of HRV studies in the literature.

Conclusions

Our selection of published primer pairs includes those from studies that have informed our current understanding of HRV epidemiology. Finding such a high degree of variability in performance was thus noteworthy. Inefficient HRV detection by PCR may be a serious problem for research studies. Comparison of data between different HRV studies is confounded as are data from studies seeking to determine the effects of other respiratory viruses. The prevalence, seasonality, transmission, and clinical effects of HRV types and species require reexamination with tools that have been comparatively validated to ensure their sensitivity.
This study was supported by the National Health and Medical Research Council, Australia, Project Grant 455905, and Queensland Children’s Medical Research Institute Research Project Seeding Grant (Established Researcher) 10281.

Ms Faux conducted this study as a research scientist in the Queensland Paediatric Infectious Diseases Laboratory, Sir Albert Sakzewski Virus Research Centre. Her main field of research was the detection and characterization of newly identified and classic respiratory viruses in children.

References


Address for correspondence: Ian M. Mackay, Queensland Children’s Medical Research Institute, Royal Children’s Hospital–Queensland Paediatric Infectious Diseases Laboratory, Sir Albert Sakzewski Virus Research Centre, Brisbane, Queensland, Australia; email: ian.mackay@uq.edu.au