March 12, 2021

*Supplemental Tables and Figures*

# Mitigating Future Respiratory Virus Pandemics: New Threats and Approaches to Consider

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**Table S1.** Published studies reporting human metapneumovirus (hMPV) related mortalities.

| Source | Time Frame of Sample Collection | Cases of hMPV identified | Deaths attributed to hMPV | Country |
|---|---|---|---|---|
| Englund JA, Boeckh M, Kuypers J, et al. Brief communication: Fatal human metapneumovirus infection in stem-cell transplant recipients. *Ann Intern Med*. 2006;144(5):344-349. | 1995 – 1999 | 5 | 4 | -- |
| Pelletier G, Déry P, Abed Y, Boivin G. Respiratory tract reinfections by the new human Metapneumovirus in an immunocompromised child. *Emerg Infect Dis*. 2002;8(9):976-978. doi:10.3201/eid0809.020238 | 1999 | 1 | 1 | Canada |
| Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human metapneumovirus infections in young and elderly adults. *J Infect Dis*. 2003;187(5):785-790. doi:10.1086/367901 | 1999 – 2001 | 44 | 1 | US |
| Walsh EE, Peterson DR, Falsey AR. Human Metapneumovirus Infections in Adults: Another Piece of the Puzzle. *Arch Intern Med.* 2008;168(22):2489–2496. doi:10.1001/archinte.168.22.2489 | 1999 - 2003 | 241 | 6 | US |
| Williams JV, Martino R, Rabella N, et al. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. *J Infect Dis*. 2005;192(6):1061-1065. doi:10.1086/432732 | 1999 – 2004 | 22 | 3 | Spain |
| Cane PA, van den Hoogen BG, Chakrabarti S, Fegan CD, Osterhaus AD. Human metapneumovirus in a haematopoietic stem cell transplant recipient with fatal lower respiratory tract disease. *Bone Marrow Transplant*. 2003;31(4):309-310. doi:10.1038/sj.bmt.1703849 | 2000 | 1 | 1 | -- |
| Morrow BM, Hatherill M, Smuts HE, Yeats J, Pitcher R, Argent AC. Clinical course of hospitalised children infected with human metapneumovirus and respiratory syncytial virus. *J Paediatr Child Health*. 2006;42(4):174-178. doi:10.1111/j.1440-1754.2006.00825.x | 2001 – 2003 | 17 | 3 | South Africa |
| Noyola DE, Alpuche-Solís AG, Herrera-Díaz A, Soria-Guerra RE, Sánchez-Alvarado J, López-Revilla R. Human metapneumovirus infections in Mexico: epidemiological and clinical characteristics. *J Med Microbiol*. 2005;54(Pt 10):969-974. doi:10.1099/jmm.0.46052-0 | 2002 – 2004 | 34 | 1 | Mexico |
| Larcher C, Geltner C, Fischer H, Nachbaur D, Müller LC, Huemer HP. Human metapneumovirus infection in lung transplant recipients: clinical presentation and epidemiology. *J Heart Lung Transplant*. 2005;24(11):1891-1901. doi:10.1016/j.healun.2005.02.014 | 2003 – 2004 | 43 | 3 | Austria |

| | | | | |
|---|---|---|---|---|
| Sivaprakasam V, Collins TC, Aitken C, Carman WF. Life-threatening human metapneumovirus infections in West of Scotland. *J Clin Virol*. 2007;39(3):234-237. doi:10.1016/j.jcv.2007.03.011 | 2004 – 2006 | 206 | 2 | Scotland |
| Boivin G, De Serres G, Hamelin ME, et al. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. *Clin Infect Dis*. 2007;44(9):1152-1158. doi:10.1086/513204 | 2006 | 6 | 3 | Canada |
| Yong Kwan Lim, Oh Joo Kweon, Hye Ryoun Kim, Tae-Hyoung Kim, Mi-Kyung Lee, Clinical Features, Epidemiology, and Climatic Impact of Genotype-specific Human Metapneumovirus Infections: Long-term Surveillance of Hospitalized Patients in South Korea, Clinical Infectious Diseases, Volume 70, Issue 12, 15 June 2020, Pages 2683 - 2694, https://doi.org/10.1093/cid/ciz697 | 2007 - 2016 | 1,275 | 7 | South Korea |
| Shahda S, Carlos WG, Kiel PJ, Khan BA, Hage CA. The human metapneumovirus: a case series and review of the literature. *Transpl Infect Dis*. 2011;13(3):324-328. doi:10.1111/j.1399-3062.2010.00575.x | 2008 – 2009 | 9 | 2 | US |
| Holzemer NF, Hasvold JJ, Pohl KJ, Ashbrook MJ, Meert KL, Quasney MW. Human Metapneumovirus Infection in Hospitalized Children. *Respir Care*. 2020;65(5):650-657. doi:10.4187/respcare.07156 | 2009 – 2013 | 131 | 1 | US |
| Liao RS, Appelgate DM, Pelz RK. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility for the elderly in Oregon. *J Clin Virol*. 2012;53(2):171-173. doi:10.1016/j.jcv.2011.10.010 | 2011 | 6 | 2 | US |
| Shih, Hsin-I MD, MPH; Wang, Hsuan-Chen MS; Su, Ih-Jen MD, PhD; Hsu, Hsiang-Chin MD; Wang, Jen-Ren PhD; Sun, Hsiao Fang Sunny PhD; Chou, Chien-Hsuan MD; Ko, Wen-Chien MD; Hsieh, Ming-I BS; Wu, Chi-Jung MD, PhD Viral Respiratory Tract Infections in Adult Patients Attending Outpatient and Emergency Departments, Taiwan, 2012–2013, Medicine: September 2015 - Volume 94 - Issue 38 - p e1545, doi: 10.1097/MD.0000000000001545 | 2012 – 2013 | 10 | 1 | Taiwan |

**Table S2.** Published studies reporting Rhinovirus Group C (HRV-C) related mortalities.

| Source | Time Frame of Sample Collection | Cases of HRV-C identified | Deaths attributed to HRV-C | Country |
|---|---|---|---|---|
| Miller EK, Edwards KM, Weinberg GA, et al. A novel group of rhinoviruses is associated with asthma hospitalizations. *J Allergy Clin Immunol*. 2009;123(1):98-104.e1. doi:10.1016/j.jaci.2008.10.007 | 2001 – 2003 | 77 | 1 | USA |
| Miller EK, Khuri-Bulos N, Williams JV, et al. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. *J Clin Virol*. 2009;46(1):85-89. doi:10.1016/j.jcv.2009.06.007 | 2007 | 62 | 1 | Jordan |
| Fuji N, Suzuki A, Lupisan S, et al. Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines. *PLoS One*. 2011;6(11):e27247. doi:10.1371/journal.pone.0027247 | 2008 – 2009 | 83 | 5 | Philippines |

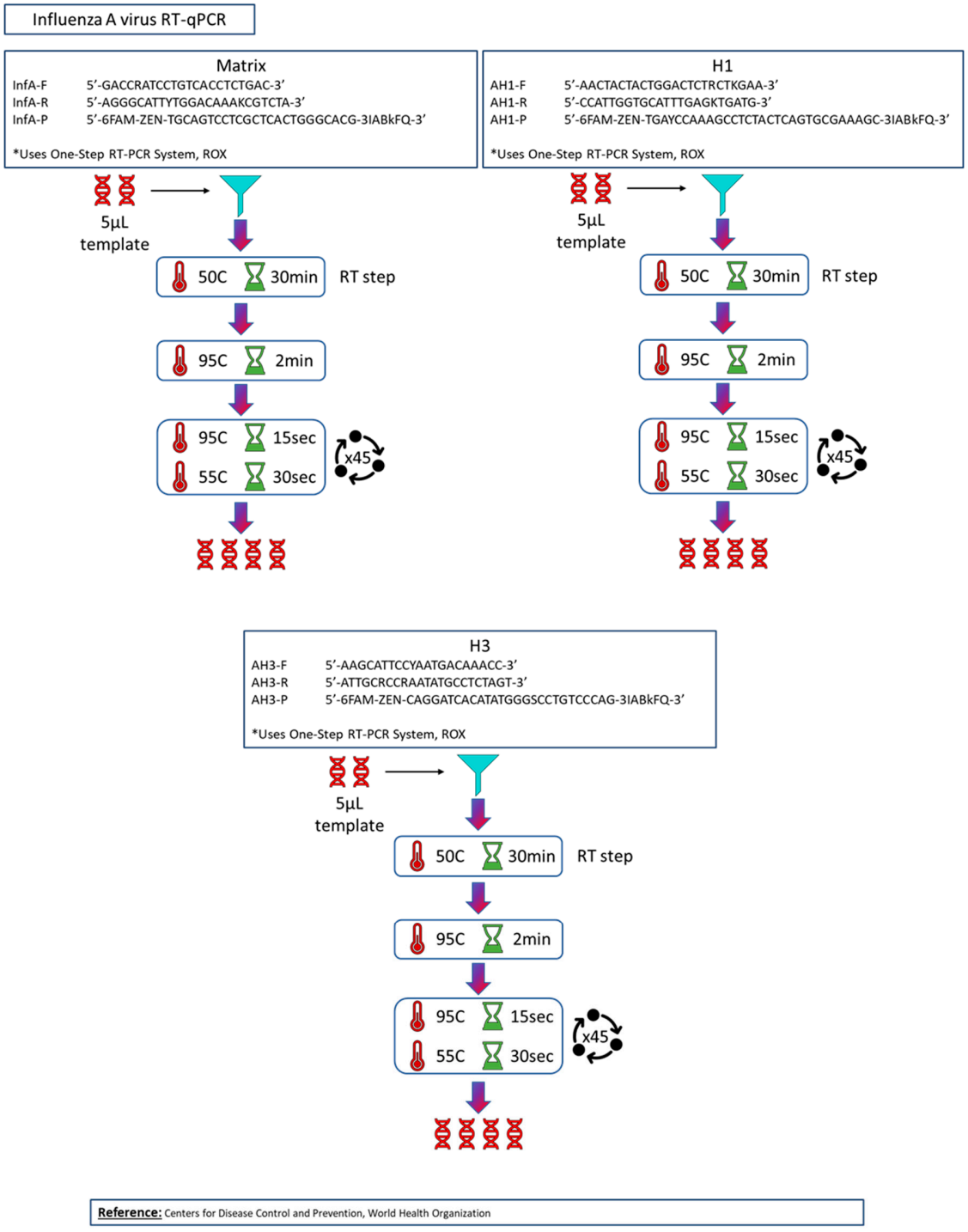



**Figure S1.** Detection of influenza A virus using reverse transcription (RT), real-time PCR (qPCR). The primers and probe specific to the matrix gene and primers and probes used for the detection of H1 and H3 (subtyping specific influenza A viruses) are shown. This protocol utilized the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen). In this assay, 0.4µL forward/reverse primers (40µM), 0.4µL probe (10µM), 0.4µL ROX (1:10 dilution), and 5µL extracted RNA are added to the qPCR reagents. The total reaction volume is 20µL.

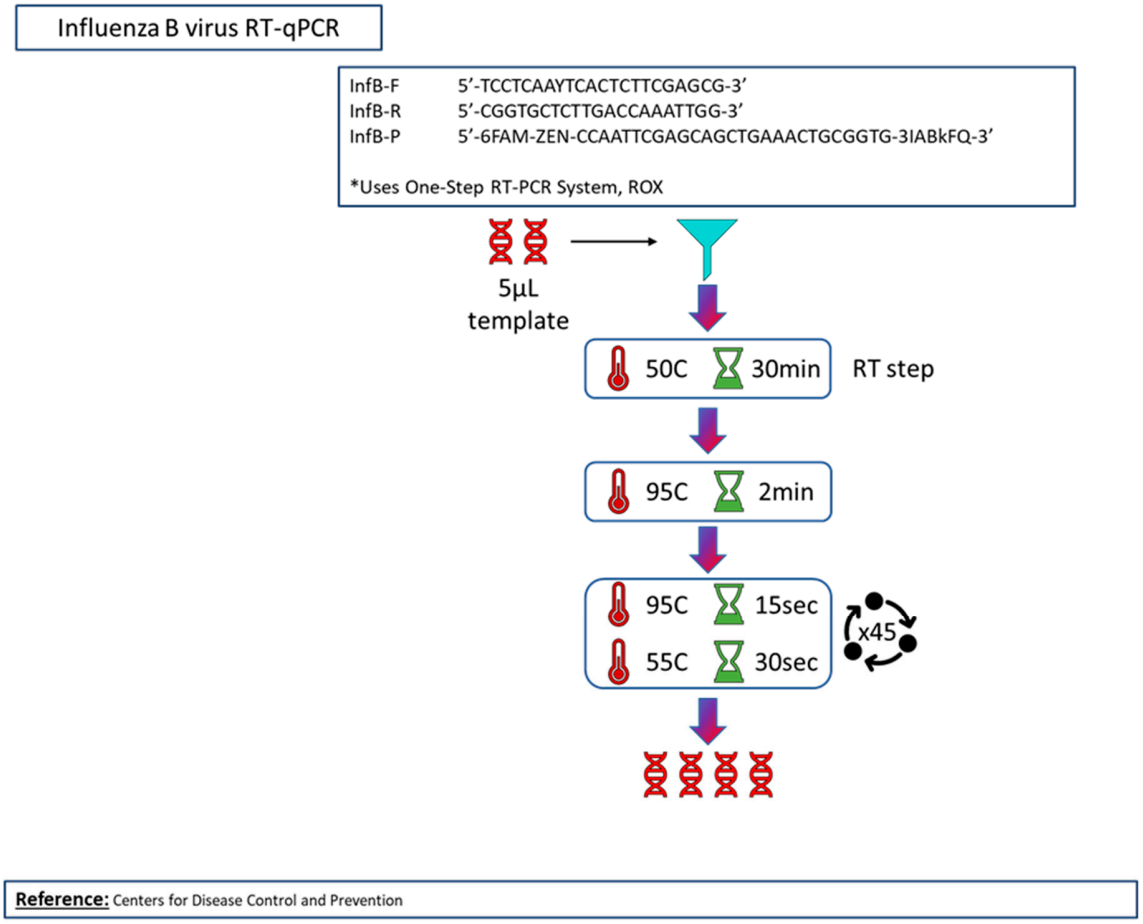



**Figure S2.** Detection of influenza B virus using reverse transcription (RT), real-time PCR (qPCR). Primers and probe specific to influenza B virus are listed. This protocol utilizes the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen). In this assay, 0.4µL forward/reverse primers (40µM), 0.4µL probe (10µM), 0.4µL ROX (1:10 dilution), and 5µL extracted RNA are added to the qPCR reagents. The total reaction volume is 20µL.

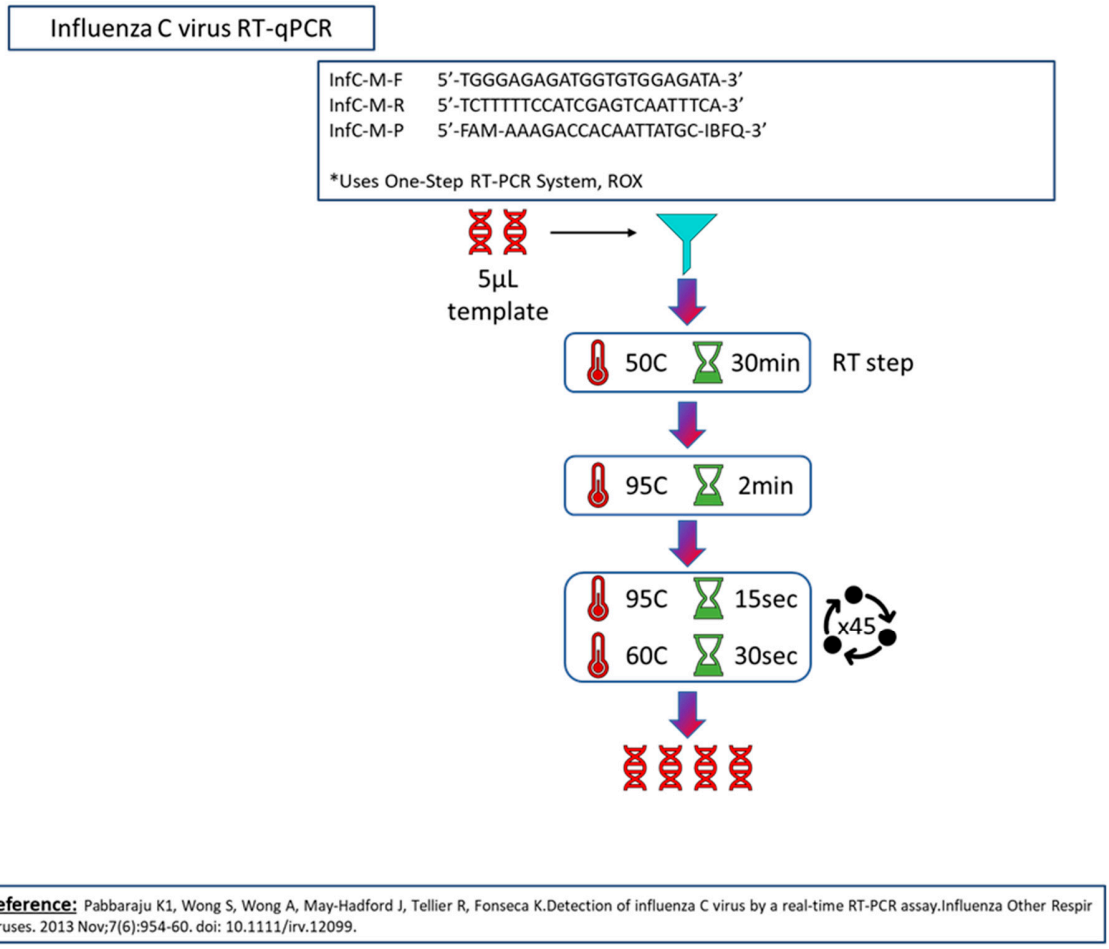



**Figure S3.** Detection of influenza C virus using reverse transcription (RT), real-time PCR (qPCR) [1]. Primers and probe specific to influenza C virus matrix protein are listed. This protocol utilizes the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen). In this assay, 0.4µL primers (40µM), 0.4µL probe (10µM), 0.4µL ROX (1:10 dilution), and 5µL extracted RNA are added to the qPCR reagents. The total reaction volume is 20µL.

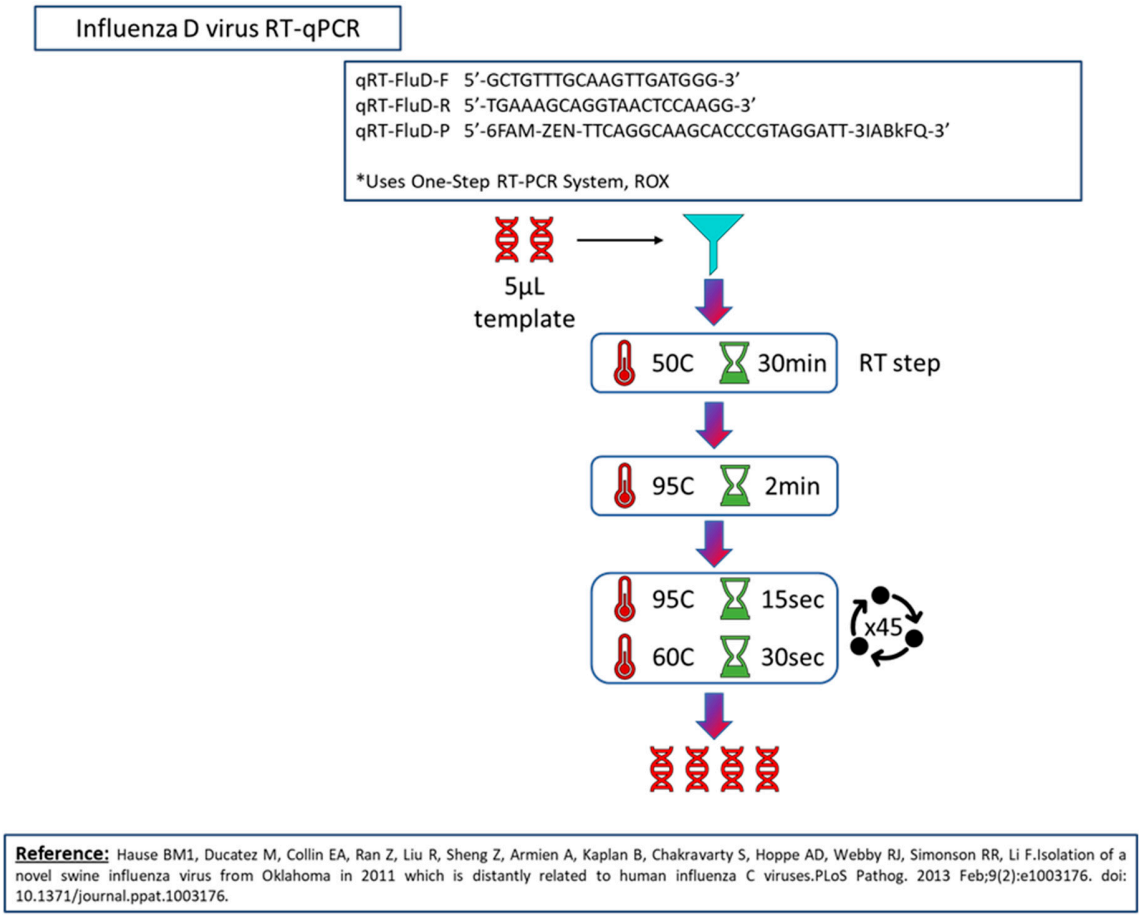



**Figure S4.** Detection of influenza D virus using reverse transcription (RT), real-time PCR (qPCR) [2]. Primers and probe specific to influenza D virus PB1 are listed. This protocol utilizes the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen). In this assay, 0.4µL primers (40µM), 0.4µL probe (10µM), 0.4µL ROX (1:10 dilution), and 5µL extracted RNA are added to the qPCR reagents. The total reaction volume is 20µL.

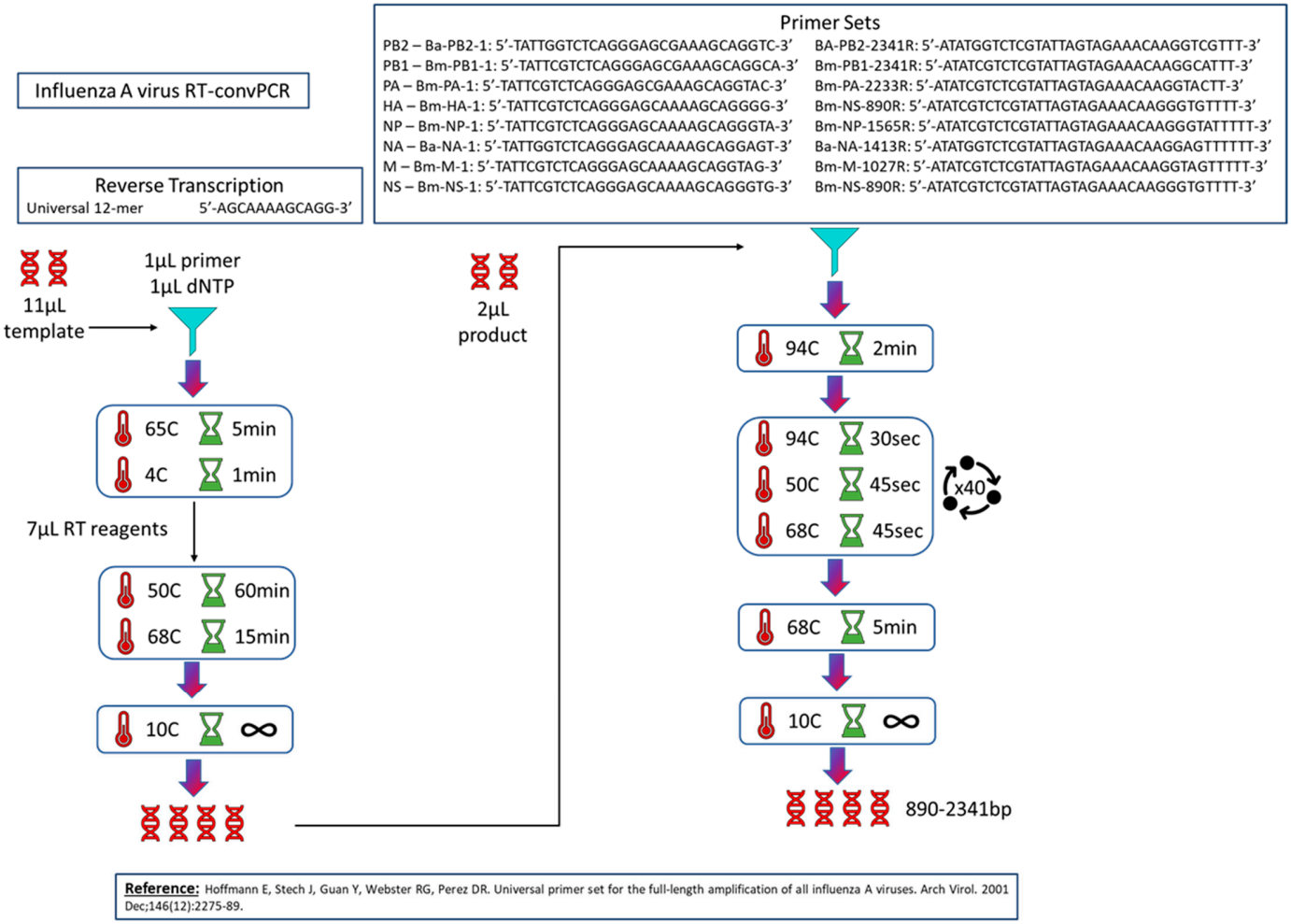



**Figure S5.** Detection of influenza A virus using reverse transcription (RT), conventional PCR (convPCR) against all 8 RNA gene segments [3]. The RT step utilizes a universal 12-mer primer. The primers specific to influenza A virus proteins for convPCR are listed. In the RT step, 1µL dNTPs (10mM) and 1µL RT primer (50µM) are mixed with 11µL extracted RNA. To this, 7µL of SuperScript III RT reagents (Invitrogen) are added. Finally, 2µL of the RT product is combined with 1µL forward/reverse primers (10µM) and Platinum Taq DNA Polymerase reagents (Invitrogen). The total volume of the PCR reaction is 50µL.

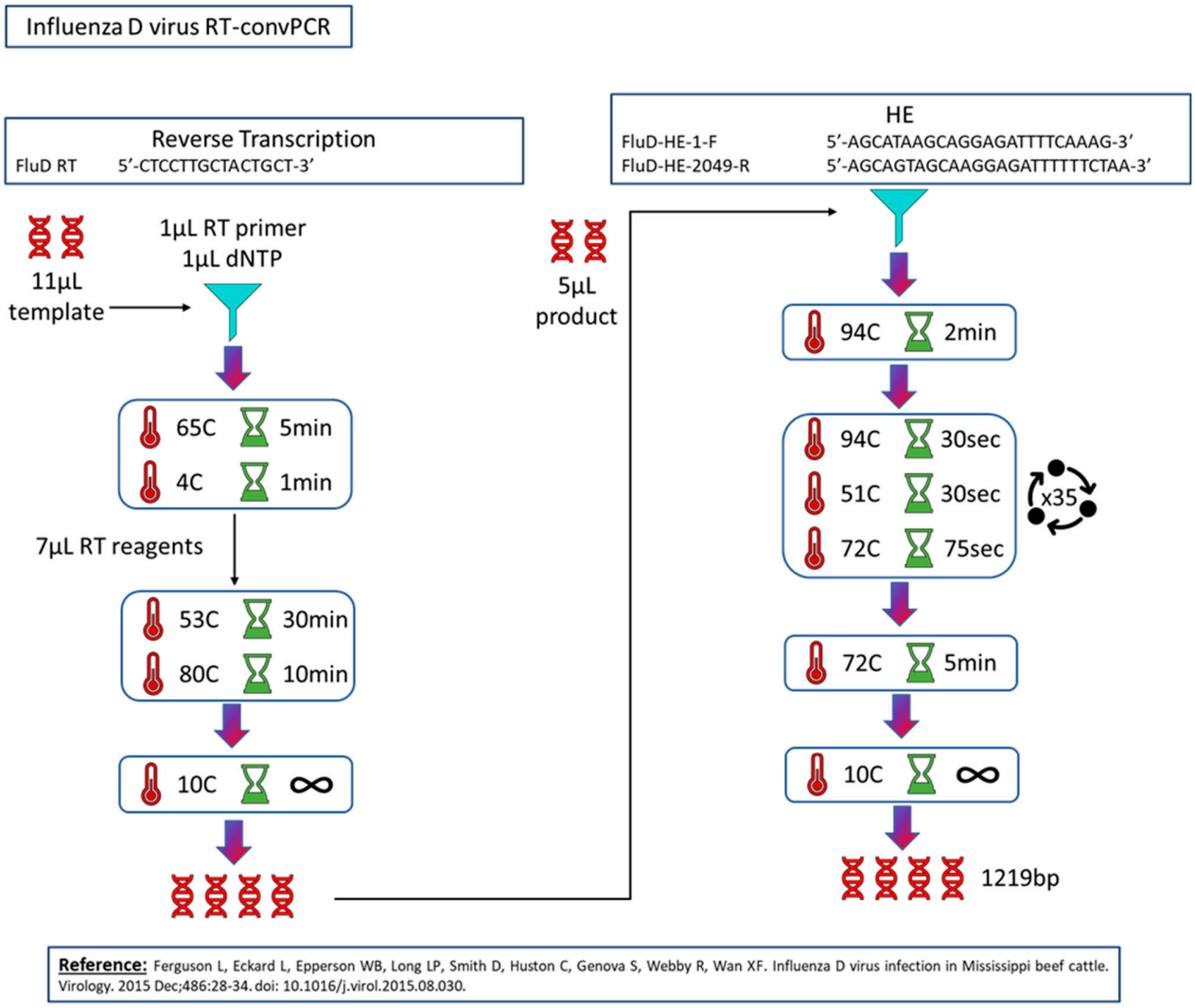



**Figure S6.** Detection of influenza D virus using reverse transcription (RT), conventional PCR (convPCR) [4]. The RT step utilizes a specific influenza D virus primer. The primers recognizing influenza D virus HE protein are listed. In the RT step, 1µL dNTPs (10mM), 1µL RT primer (2µM), and 2µL water are mixed with 11µL extracted RNA. To this, 7µL of SuperScript IV RT reagents (Invitrogen) are added. Finally, 5µL of the RT product is combined with 0.5µL forward/reverse primers (10µM) and Platinum Taq DNA Polymerase reagents (Invitrogen). The total volume of the reaction is 25µL.

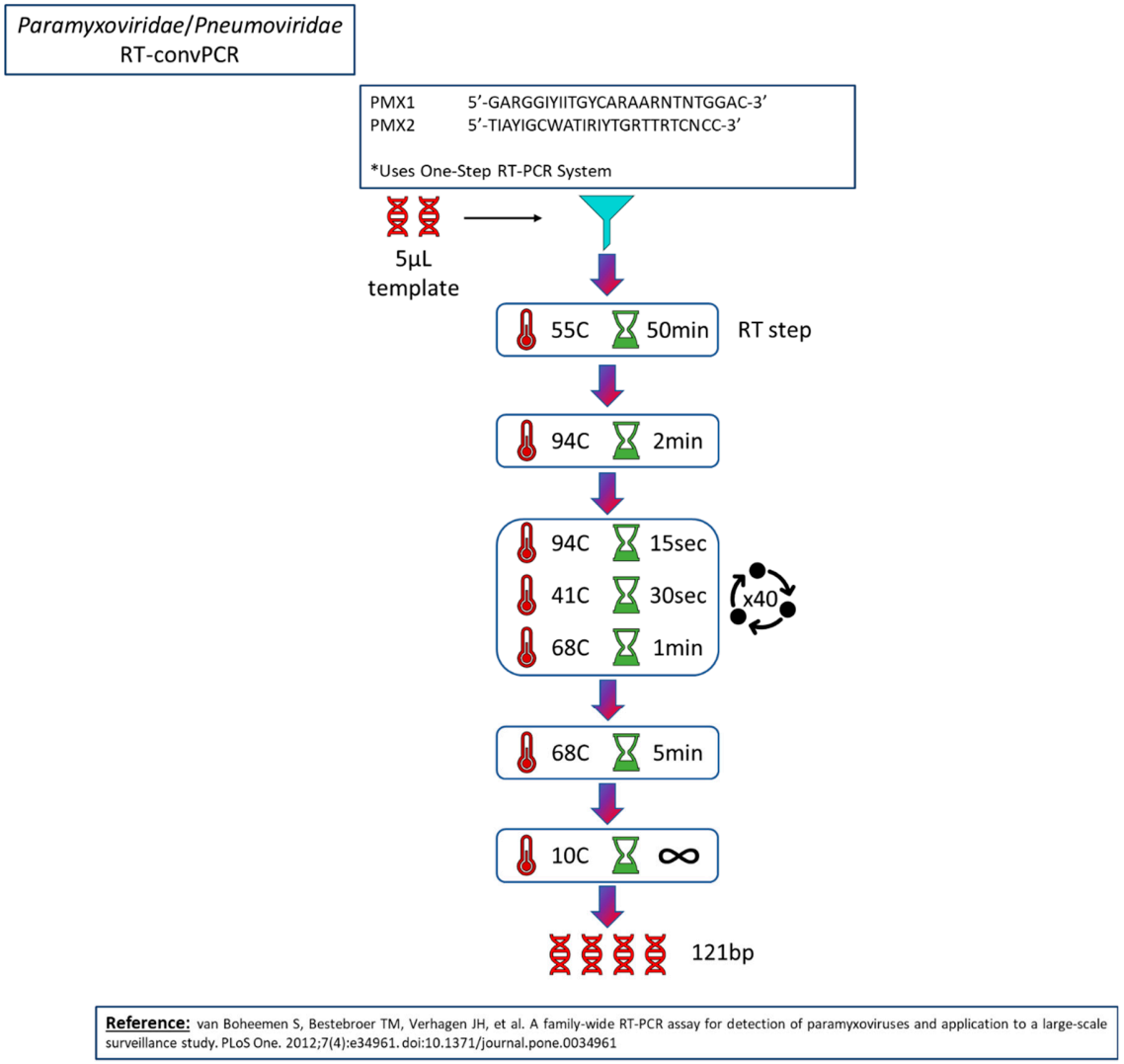



**Figure S7.** Detection of *Paramyxoviridae/Pneumoviridae* viruses using reverse transcription (RT), conventional PCR (convPCR) [5]. The RT step utilizes the forward/reverse primers. The primers recognizing paramyxovirus are listed. This assay utilizes the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen). The RT-PCR reagents are combined with 1µL forward/reverse primers (10µM) and 5µL extracted RNA. The total volume of the reaction is 25µL.

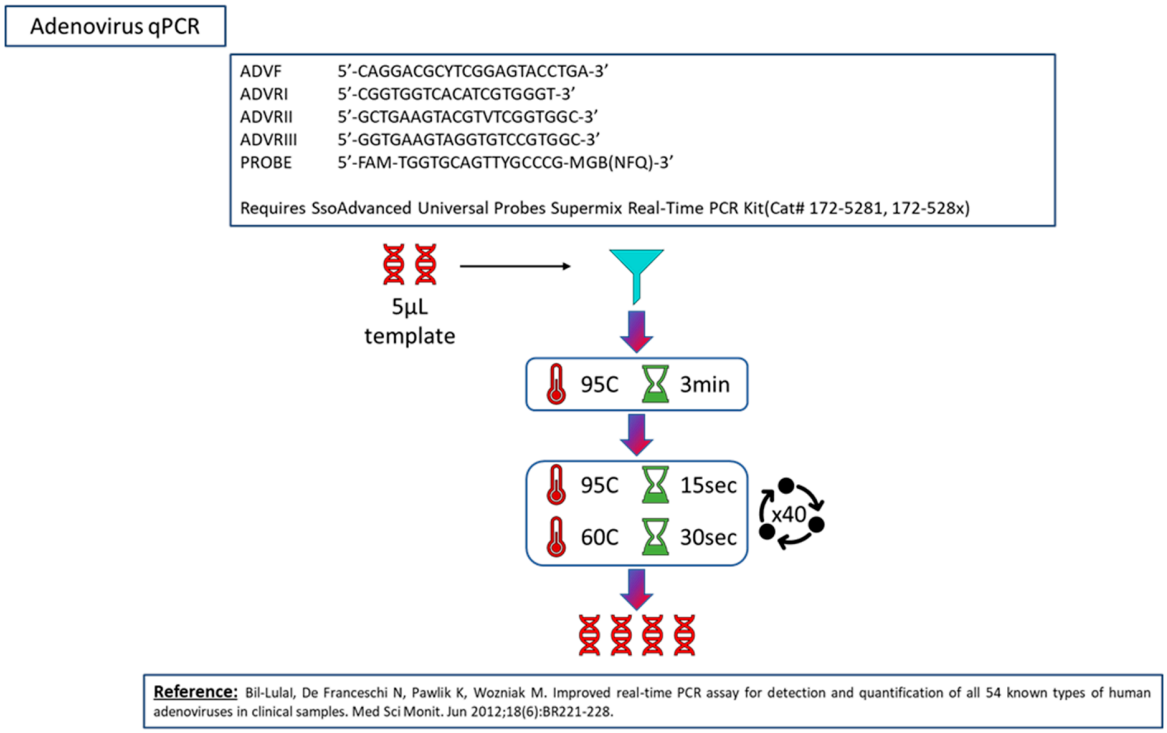



**Figure S8.** Detection of human adenoviruses using real-time PCR (qPCR) [6]. The primers and probe recognizing adenovirus are listed and include three reverse primers. This assay utilizes the SsoAdvanced Universal Probes Supermix Real-Time PCR kit (Bio-Rad Laboratories). To the supermix, 0.6µL forward primer (10µM), 0.7µL reverse primer I (25µM), 0.7µL reverse primer II (25µM), 0.6µL reverse primer III (10µM), 1µL probe (5µM), and 5µL extracted DNA are added. The total volume of the reaction is 20µL.

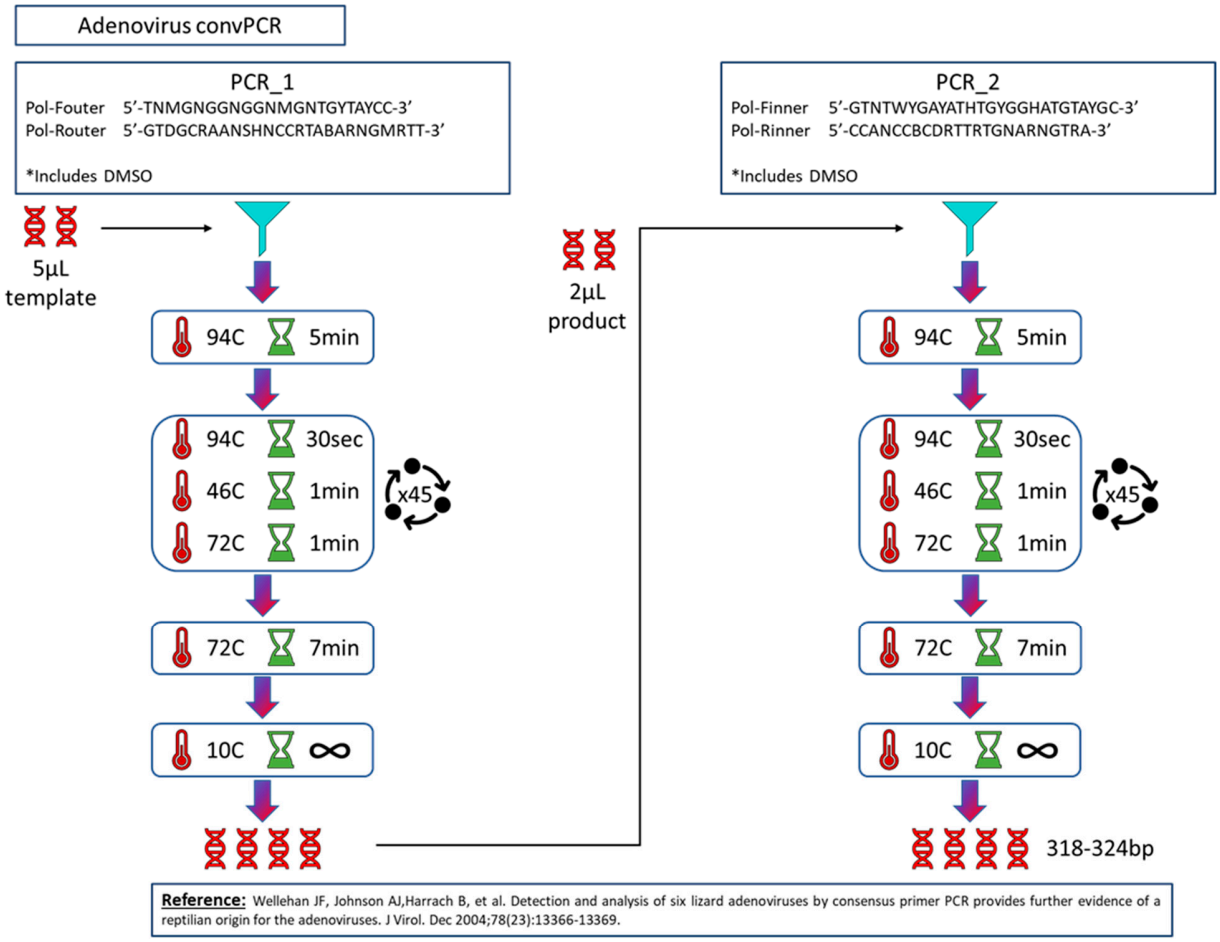



**Figure S9.** Detection of animal adenoviruses using conventional PCR (convPCR) [7]. The PCR 1 and PCR 2 primers recognizing adenovirus are listed. This assay utilizes the Platinum Taq DNA Polymerase kit (Invitrogen). For PCR 1, 0.5µL forward primer (25µM), 0.5µL reverse primer (25µM), 0.5µL dNTP (10mM), 0.24µL 100% dimethyl sulfoxide (DMSO), and 5µL extracted DNA are added to the PCR mix. The total volume of the reaction is 25µL. This is repeated for PCR 2 using the PCR 2 primers and 2µL of PCR 1 product.

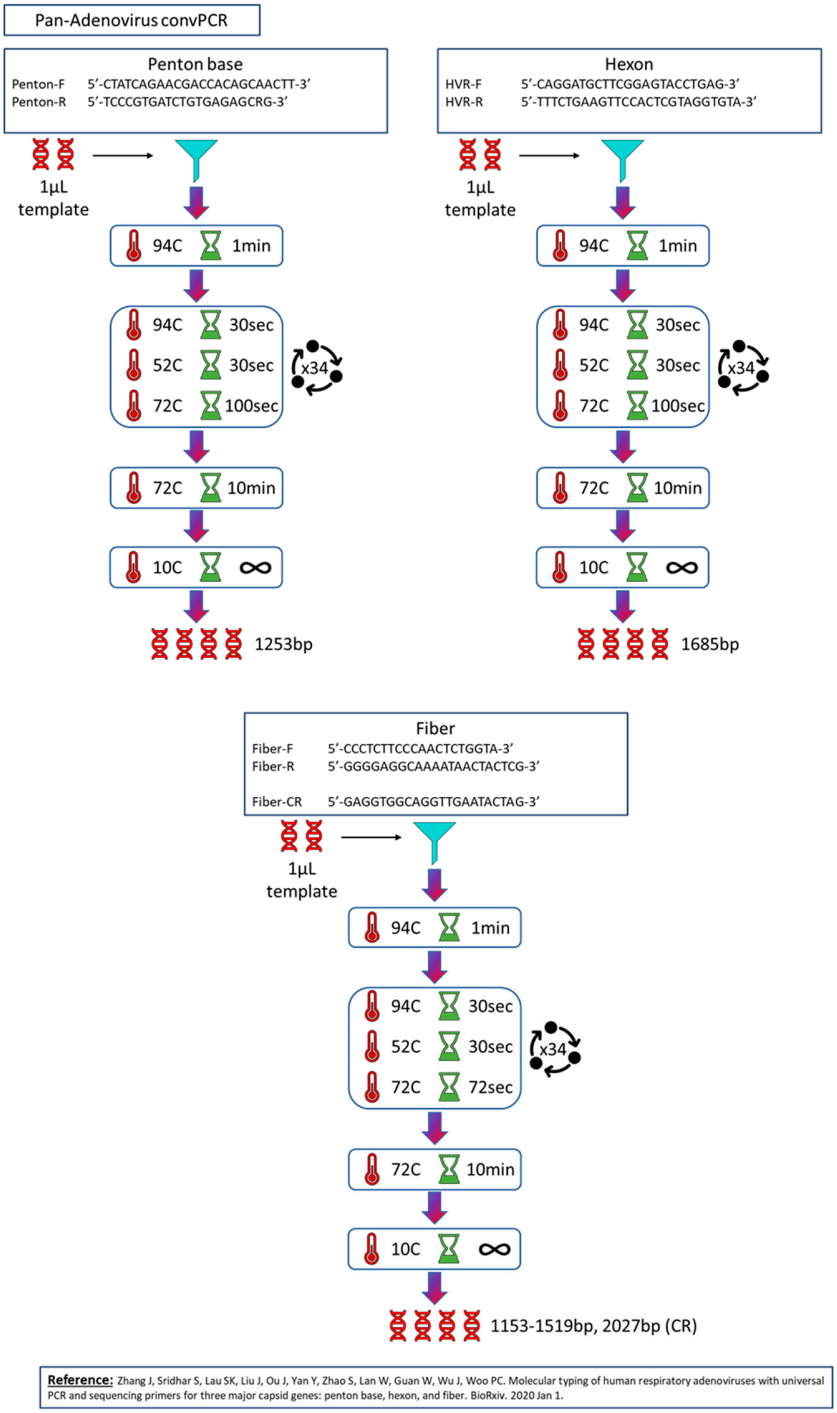



**Figure S10.** Detection of pan-adenoviruses using conventional PCR (convPCR) [8]. The primers recognizing adenovirus penton base, hexon, and fiber are listed. This assay utilizes the Platinum Taq DNA Polymerase kit (Invitrogen). For each reaction, 0.5µL forward primer (10µM), 0.5µL reverse primer (10µM), 0.5µL dNTP (10mM), and 1µL extracted DNA are added to the PCR mix. The total volume of the reaction is 25µL. Note the different PCR running conditions for the Fiber gene.

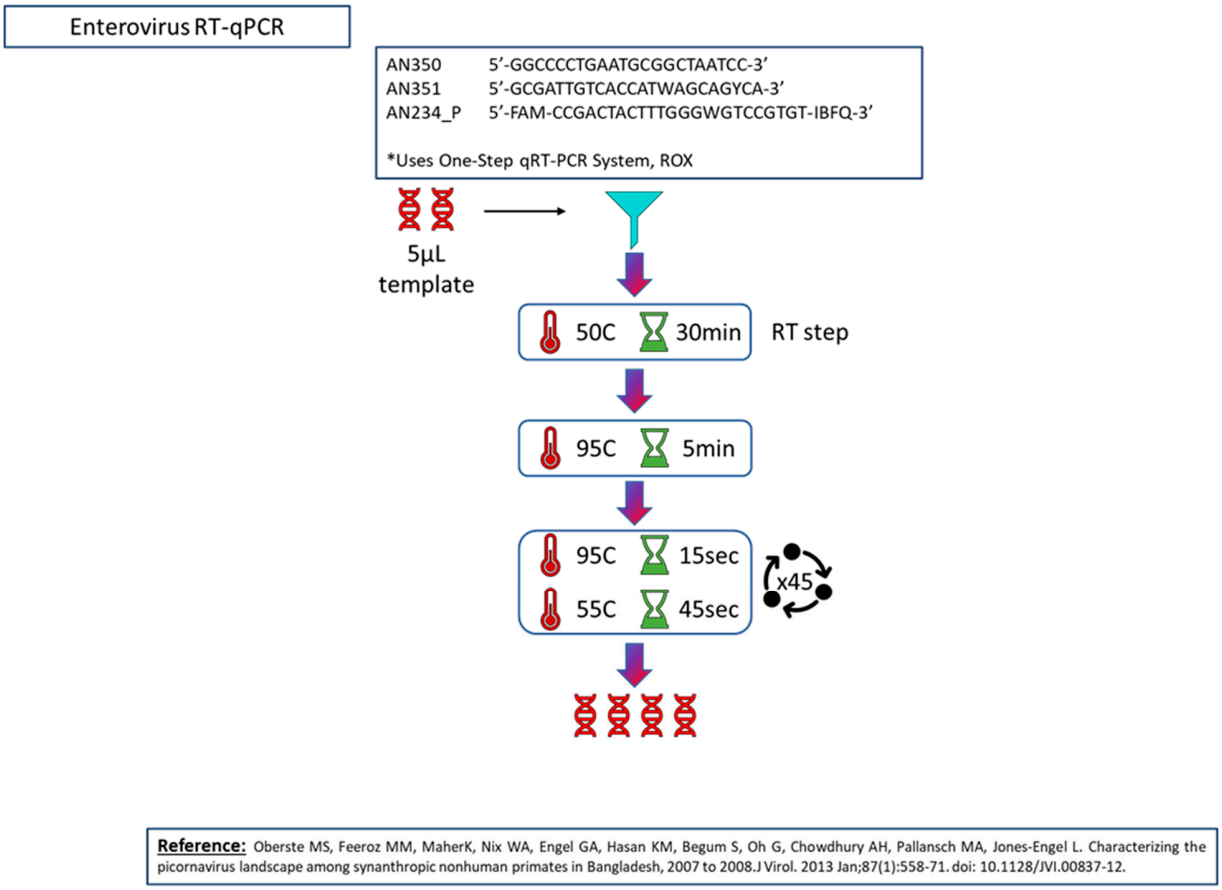



**Figure S11.** Detection of enterovirus using reverse transcription (RT), real-time PCR (qPCR) [9]. The primers and probe recognizing enterovirus are listed. This assay utilizes the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen). To the qPCR mix, 0.2µL forward primer (40µM), 0.2µL reverse primer (40µM), 0.2µL probe (10µM), 0.4µL ROX (1:10 diultion), and 5µL extracted RNA are added. The total volume of the reaction is 20µL.

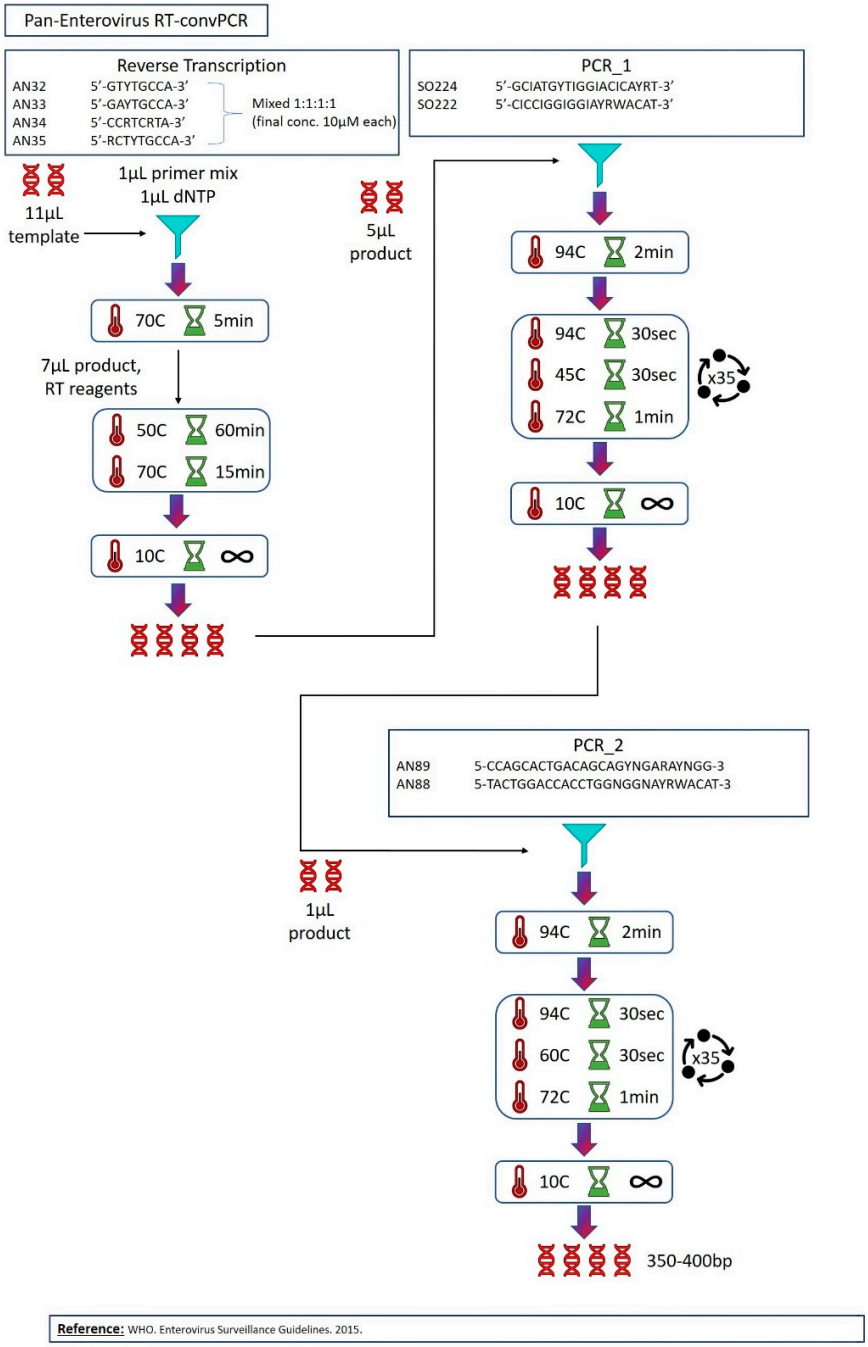
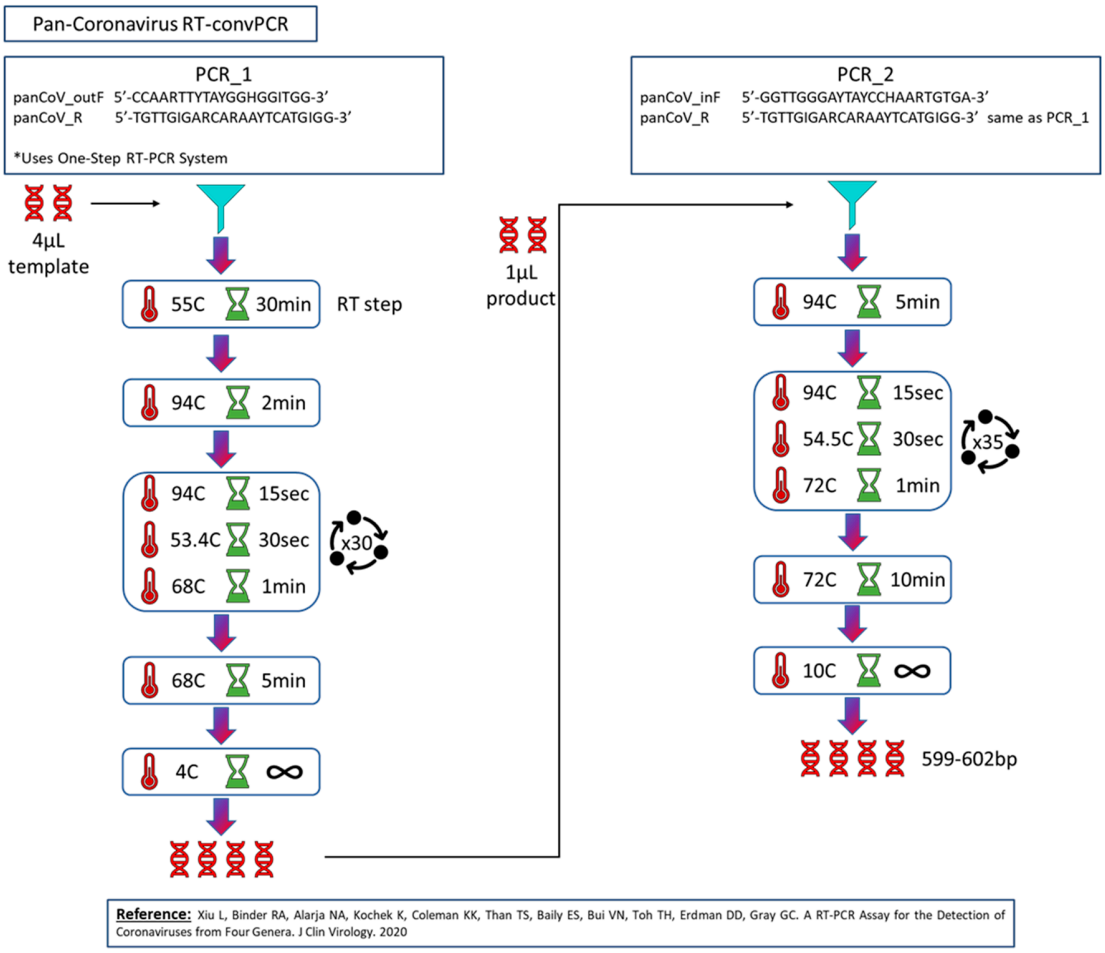
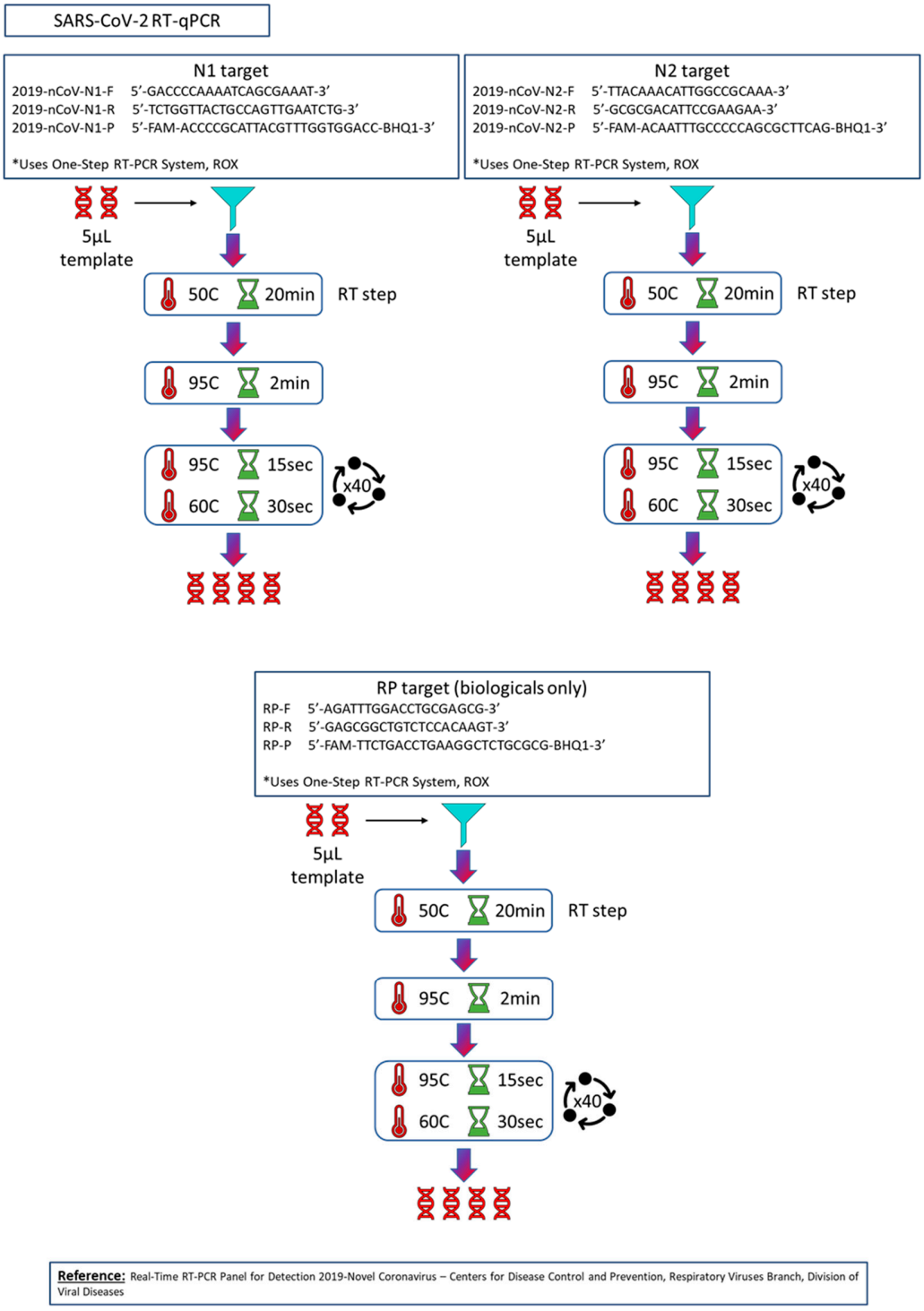



**Figure S12.** Detection of pan-enteroviruses using reverse transcription (RT), conventional PCR (convPCR) [10]. The RT step, PCR 1, and PCR 2 primers recognizing enterovirus are listed. This assay utilizes the SuperScript III RT Enzyme (Invitrogen) and Platinum Taq DNA Polymerase (Invitrogen) kits. For the RT step, the four RT primers (10µM) are combined in a 1:1:1:1 ratio. To 11µL extracted RNA, 1µL of the RT primer mix and 1µL dNTP (10mM) are added. Following incubation, 7µL of the RT reagents are added. For PCR 1, 0.5µL forward primer (10µM), 0.5µL reverse primer (10µM), 0.5µL dNTP (10mM), and 5µL DNA template are added to the PCR mix. The total volume of the reaction is 25µL. For PCR 2, 0.5µL forward primer (10µM), 0.5µL reverse primer (10µM), 0.5µL dNTP (10mM), and 1µL PCR 1 product are added to the PCR mix. The total volume of the reaction is 25µL.




**SFigure 13.** Detection of pan-coronaviruses using reverse transcription (RT), conventional PCR (convPCR) [11]. The PCR 1 and PCR 2 primers recognizing coronavirus are listed. This assay utilizes the SuperScript III OneStep RT-PCR System with Platinum Taq DNA Polymerase kit (Invitrogen) in the first step and Platinum Taq DNA Polymerase kit (Invitrogen) in the second step. For PCR 1, 1µL of the forward primer (10µM), 1µL reverse primer (10µM), and 4µL extracted RNA are added to the PCR mix. The total volume of the reaction is 25µL. For PCR 2, 1µL forward primer (10µM), 1µL reverse primer (10µM), 0.5µL dNTP (10mM), and 1µL PCR 1 product are added to the PCR mix. The total volume of the reaction is 25µL.




**SFigure 14.** Detection of SARS-CoV-2 using reverse transcription (RT), real-time PCR (qPCR) [12]. The primers and probe recognizing coronavirus N1 and N2 proteins, and RP control protein (only for human biological samples) are listed. These assays utilize the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) and 2019-nCoV CDC qPCR Probe Assay primer/probe mix (IDT). To the qPCR mix, 1.5µL N1/N2/RP primer/probe mix, 0.4µL ROX (1:10 diultion), and 5µL extracted RNA are added. The total volume of the reaction is 20µL.

## References


1. Pabbaraju, K.; Wong, S.; Wong, A.; May-Hadford, J.; Tellier, R.; Fonseca, K., Detection of influenza C virus by a real-time RT-PCR assay. *Influenza Other Respir Viruses* **2013,** 7, (6), 954-60.
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4. Ferguson, L.; Eckard, L.; Epperson, W. B.; Long, L. P.; Smith, D.; Huston, C.; Genova, S.; Webby, R.; Wan, X. F., Influenza D virus infection in Mississippi beef cattle. *Virology* **2015,** 486, 28-34.
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6. Bil-Lula, I.; De Franceschi, N.; Pawlik, K.; Mieczyslaw, W., Improved real-time PCR assay for detection and quantification of all 54 known types of human adenoviruses in clinical samples. *Medical Science Monitor* **2012,** 18, (6), 221-228.
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9. Oberste, M. S.; Feeroz, M. M.; Maher, K.; Nix, W. A.; Engel, G. A.; Hasan, K. M.; Begum, S.; Oh, G.; Chowdhury, A. H.; Pallansch, M. A.; Jones-Engel, L., Characterizing the picornavirus landscape among synanthropic nonhuman primates in Bangladesh, 2007 to 2008. *J Virol* **2013,** 87, (1), 558-71.
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12. Centers for Disease Control and Prevention *CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel*; Centers for Disease Control and Prevention, Division of Viral Diseases: 2020.