Deteksi patogen infeksi saluran pernapasan akut saat Pandemi COVID-19 (Maret sampai September 2020) = Detection of acute respiratory tract infection pathogens during the COVID-19 Pandemic (March to September 2020)

Simon Yosonegoro Liem, author

Abstrak

Latar Belakang: Infeksi saluran napas akut (ISPA) disebabkan oleh berbagai patogen termasuk SARS-CoV-2. Gejala yang disebabkan oleh SARS-CoV-2 maupun patogen lainnya memiliki kemiripan sehingga diperlukan uji deteksi SARS-CoV-2 terkait tatalaksana infeksi SARS-CoV-2 yang di antaranya diperlukan isolasi kasus positif. Tujuan penelitian ini adalah untuk mendeteksi patogen penyebab ISPA selama pandemi COVID-19 sehingga dapat dilakukan penatalaksanaan dengan cepat dan tepat.
Metode: Pada tahap awal dilakukan optimasi kondisi real-time reverse-transcription- PCR (rRT-PCR) menggunakan kontrol positif SARS-CoV-2 dan kit SensiFAST SYBR No-ROX One-Step dengan parameter yakni volume template, suhu reverse transcriptase (RT), dan waktu RT, serta dilakukan uji Limit of Detection (LOD) dengan pengenceran RNA standar genom SARS-CoV-2 dan uji reaksi silang rRT-PCR SARS-CoV-2 terhadap 11 jenis bakteri dan 4 jenis virus. Setelah optimasi, dilakukan uji terhadap spesimen klinis (swab nasofaring/orofaring) yang dikumpulkan dari Maret sampai September 2020. RNA spesimen diekstraksi menggunakan QIAamp® Viral RNA Mini Kit dan kemudian digunakan untuk rRT-PCR. Patogen penyebab ISPA lainnya (Virus Influenza, Adenovirus, Bocavirus, Coronavirus (229E, HKU-1, NL63, OC43), Metapneumovirus, Virus Parainfluenza, Respiratory Syncytial Virus (RSV), Rhinovirus, Bordetella pertussis, B. parapertussis, dan Mycoplasma pneumonia) dideteksi menggunakan Hybrispot Respiratory Flow Chip assay.
Hasil: Kondisi optimal rRT-PCR SARS-CoV-2 didapatkan volume template 7,9 L, suhu RT 50 oC, waktu RT 30 menit dengan LOD 3,5 kopi/reaksi, dan tidak ditemukan reaksi silang dengan patogen lainnya yang diuji. Dari 261 spesimen klinis, diperoleh hasil yang positif minimal satu patogen sebesar 27,59% (72/261) dengan 15 jenis patogen penyebab ISPA yang terdiri dari 13 spesies/subtipe virus yakni SARS-CoV-2, Coronavirus-229E (CoV-229E), CoV-HKU-1, CoV-NL 63, CoV-OC43, Adenovirus, Bocavirus, Virus Influenza, Human Metapneumovirus, Respiratory Syncytial Virus (RSV) subtipe-A, RSV subtipe-B, Rhinovirus, dan 2 spesies bakteri yakni Bordetella pertussis dan B. parapertussis.
Kesimpulan: Uji rRT-PCR dapat dengan akurat dan cepat mendeteksi SARS-CoV-2 sehingga dapat dilakukan penatalaksanaan secara tepat dan cepat untuk mencegah penularan COVID-19. Gejala klinis (demam, batuk, pilek, nyeri tenggorokan, sesak napas, nyeri abdomen dan diare) pada pasien yang terinfeksi SARS-CoV-2 dengan pasien yang terinfeksi patogen lainnya tidak dapat dibedakan; namun perlu dilakukan studi lebih lanjut menggunakan jumlah sampel yang memenuhi kriteria statistik untuk membedakan gejala infeksi SARS-CoV-2 dengan infeksi patogen lainnya.
.....Background: Acute respiratory tract infections (ARTI) are caused by various pathogens, including SARS-CoV-2. The symptoms caused by SARS-CoV-2 and other pathogens are similar, so a SARS-CoV-2 detection test is needed to manage SARS- CoV-2 infection, including the isolation of positive cases. The purpose of this study is to detect pathogens that cause ARTI during the COVID-19 pandemic so that

management can be carried out quickly and appropriately.
Method: The first step of this research was an optimization of real-time reverse- transcription-PCR (rRT-PCR) using a positive control SARS-CoV-2 and the SensiFAST SYBR No-ROX One-Step kit with parameters: template volume, reverse transcriptase (RT) temperature, and RT time, as well as the Limit of Detection (LOD) test by dilution of the standard RNA of the SARS-CoV-2 genome and cross-reaction test of SARS-CoV-2 against 11 types of bacteria and 4 types of viruses. After optimization, clinical specimens (nasopharyngeal/oropharyngeal swabs) were tested, collected from March to September 2020. RNA specimens were extracted using the QIAamp® Viral RNA Mini Kit and then used for rRT-PCR. Other respiratory pathogens (Influenza Virus, Adenovirus, Bocavirus, Coronavirus (229E, HKU-1, NL63, OC43), Metapneumovirus, Parainfluenza Virus, Respiratory Syncytial Virus (RSV), Rhinovirus, Bordetella pertussis, B. parapertussis, and Mycoplasma pneumoniae) were detected using the Hybrispot Respiratory Flow Chip assay.
Result: The optimal conditions for SARS-CoV-2 rRT-PCR were 7.9 L template volume, 50 oC RT temperature, 30 minutes RT time with LOD of 3.5 copies/reaction, and no cross-reactions were found with other tested pathogens. Positive clinical specimen for at least one pathogen was 27.59% (72/261) with 15 types of ARTI pathogens consisting of 13 species/subtypes of the virus, i.e., SARS-CoV-2, Coronavirus-229E (CoV-229E), CoV-HKU-1, CoV-NL 63, CoV-OC43, Adenovirus, Bocavirus, Influenza Virus, Human Metapneumovirus, Respiratory Syncytial Virus (RSV) subtype-A, RSV subtype-B, Rhinovirus, and 2 bacterial species, i.e., Bordetella pertussis and B. parapertussis.
Conclusion: The rRT-PCR test can detect SARS-CoV-2 accurately and quickly for appropriate and rapid management in preventing the transmission of COVID-19. The clinical symptoms (fever, cough, nasal congestion, sore throat, dyspneu, abdominal discomfort and diarrhea) of patients infected with SARS-CoV-2 compared to those infected with other pathogens were indistinguishable; however, further studies need to be conducted using the number of samples that meet the statistical criteria to differentiate the symptoms of SARS-CoV-2 infection from other pathogenic infections.