Brief report

Interpretation of borderline results with 40<Cq≦45 in real-time one-step reverse transcriptase-polymerase chain reaction（RT-PCR）for severe acute respiratory syndrome coronavirus 2（SARS-CoV-2）of coronavirus disease 2019（COVID-19）used nasopharyngeal scraped specimens to decrease its false negativity

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Background : We have diagnosed SARS-CoV-2 of COVID-19 by one-step RT-PCR with TaqMan probe to nasopharyngeal scraped specimens according to the standard of procedure（SOP）established by National institute of infectious diseases in 2020. Its sensitivity of lowest level was required at viral RNA 5 copies/μl by 45 times of PCR and, furthermore, its standard positive value was threshold cycle（Cq）≦40（50copies/μl≦）. But the increasing proliferation curve after 40 < Cq should have reviewed to rule out false negative cases. In this report we examined the number of Cq in lower viral RNA copy.

Materials and methods : The positive RNA of SARS-CoV-2 was obtained from Nihon gene research laboratories. The standard curve was examined according to RNA level from 2.5/μl to 50000/μl. Viral RNA was checked by 45 times of PCR with one-step RT-PCR with TaqMan probe（Takara SARS-CoV-2 direct detection kit）and AriaMX Real-Time PCR（Agilent）. The relationship between viral RNA level and Cq value was checked.

Results : At RNA 5 copies/μl, Cq was 35.5（mean）± 0.745（σ）, and at RNA 2.5 copies/μl, Cq 36.6 ± 1.52. On semi-logarithm graph, there were 2.1 copies/μl and 3.8 copies/μl, at 5% hazard ratio and 0.3% hazard ratio, respectively.

Conclusion : In the false-positive case unsatisfied the standardized diagnostic criteria of Cq≦40 in PCR test, we found that 2-4 viruses were present after 40 times of PCR cycles（40 < Cq）. Supposing that the doubling time of virus is eight hours, the virus shall grow up to 128-256 copies/μl after 2 days, when the PCR test shall turn to positive. Therefore, the false negative case with 40 < Cq≦45 should be intensely followed with re-examination to prevent a possibility of the nosocomial infection by patients with false-negative judgment. The above-mentioned advice shall be surely recognized by an addendum report of the amplification plot figure demonstrating late increasing plot curve pattern.

Key words : one-step reverse transcriptase-polymerase chain reaction（RT-PCR）, AriaMX（Agilent）, real-time PCR, severe acute respiratory syndrome coronavirus 2（SARS-CoV-2）, coronavirus disease 2019（COVID-19）, nasopharyngeal scraped specimens, decreasing false negativity, 40 < Cq≦45

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短報

コロナウイルス病2019（COVID-19）の重症急性呼吸症候群コロナウイルス 2（SARS-CoV-2）診断のための鼻咽頭擦過標本を使用したリアルタイム・ワンステップ逆転写酵素－ポリメラーゼ連鎖反応（RT-PCR）において、偽陰性を減少する為の40＜Cq≦45症例の対応

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　コロナウイルス病2019（COVID-19）の重篤急性呼吸症候群コロナウイルス 2（SARS-CoV-2）診断のための鼻咽頭擦過標本を使用したリアルタイム・ワンステップ逆転写酵素 - ポリメラーゼ連鎖反応（RT-PCR）において、保険収載の判定基準 Cq≦40（注意：　Cq＝Ct、増殖曲線と閾値の交点での PCR サイクル数）を満たせない40＜Cq≦45の陰性症例を単純に陰性として放置すべきではない。検量線より、40＜Cq においてウイルスが少数ながら認められることより、初回検査 2 日以降の再検査が望ましく、診断医への警告として増殖曲線図を追加添付することが有用である。

キーワード：リアルタイム・ワンステップ逆転写酵

素－ポリメラーゼ連鎖反応（RT-PCR）、AriaMX（アジレント）、コロナウイルス病2019（COVID-19）、重症急性呼吸症候群コロナウイルス 2 （SARS-CoV-2）、鼻咽頭擦過標本、偽陰性例の抑制、40<Cq≦45