Validatie

Bijlage 1. V040 Validatierapport Coronavirus 2019 n-CoV detectie PCR_met_ref

Verification of qRT-PCR assay for detection of SARS-CoV-2

Method: qPCR – One step RT-PCR with Fast Virus Master Mix
Manufacturer/Origin: Biolegio
Type of validation: verification of improved primers/probe set specific for detection of SARS-CoV-2
Application test: patient diagnostics, detection of SARS-CoV-2 in clinical material
Conclusion: Based on the results, we conclude that using the improved primers/probe set the RT-PCR meets the predefined criteria of the following parameters: measurement trueness, measurement accuracy, quantitation limit, analytical sensitivity and detection limit. The RT-PCR is more sensitive than with the original primers/probe set and now has similar sensitivity as the E-gene RT-PCR. Therefore the improved primers/probe set has been accepted for implementation in routine diagnostics of SARS-CoV-2.
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Introduction & purpose

At this moment when a request is made for corona diagnostics the sample is tested on 2 genes of SARS-CoV-2. The E-gene and RdRp-gene are detected in 2 separate PCRs. The E-gene PCR is more sensitive than the RdRp-gene PCR. With an adjustment of the primers and probe set for the RdRp-gene, the idea is that the sensitivity of RdRp-gene PCR improves, so

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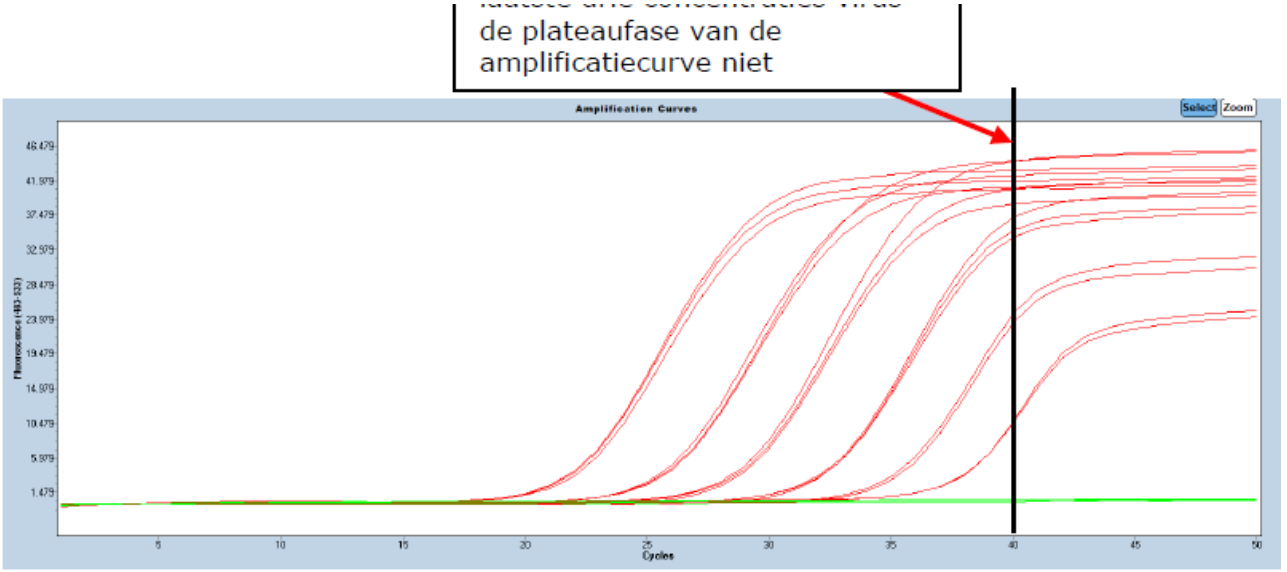

Figuur. Amplificatiecurves van RdRP-gen van 10-voudige verdunningsreeks SARS-CoV-2 gesimuleerde klinische monsters in drievoud getest (rood) in de achtergrond van negatieve klinische monsters (groen). De laatste curves rechts zijn bij een concentratie virus op de detectiegrens van de PCR-test waarbij de PCR-test in 2 van de 3 herhalingen een amplificatiecurve geeft. Zou 40 cycli gedraaid zijn ipv 50 cycli dan zouden de laatste curves voordat ze de S-vorm krijgen afgekapt zijn. Dat geldt ook voor de 10-voudig en 100-voudig hogere concentraties daarvoor. 50 cycli draaien maakt het beoordelen van de curves een stuk gemakkelijker. Bij minder dan 40 cycli draaien zou er sensitiviteit verlies optreden.

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Analytic parameters:

The detection qPCR is not used as a pure quantitative method. Results are scored positive/negative to confirm if a patient is infected with SARS-CoV-2. Ct values are used as semi-quantitative information when useful for interpretation of the result. Only those parameters are tested that need verification compared to the original validation

Measurement uncertainty (Meetonzekerheid):	– not tested
Measurement trueness (Juistheid):	– tested: Saliva panel (page 6)
Measurement accuracy (Accuraatheid):	– tested: Saliva panel (page 6)
Measurement precision including measurement repeatability (Precisie inclusief herhaalbaarheid):	– not tested
Analytical sensitivity (Analytische sensitiviteit):	– tested: LOD95 determination (page 4)
Analytical specificity, including interfering substances (Analytische specificiteit met inbegrip van interfererende substanties):	– not tested
Detection limit (Detectielimiet, bij kwantitatieve methoden):	– tested: LOD95 determination (page 4)
Quantitation limit (Kwantificatielimiet, bij kwantitatieve methoden):	– tested: 10-fold dilution of quantified control (page 2)
Measuring interval (Meetinterval, kwantitatieve methoden):	– not tested

Clinical parameters:

Diagnostic sensitivity (Diagnostische Sensitiviteit):	– not tested
Diagnostic specificity (Diagnostische Specificiteit):	– not tested