The Takara SARS-CoV-2 direct PCR detection kit delivers reliable results with throat wash specimens

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Dear Editor,

Actually, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection by quantitative polymerase chain reaction (qPCR) still represents the diagnostic gold standard regarding the global SARS-CoV-2 pandemic. Nevertheless, qPCR diagnostic is a rather time-consuming process depending on nucleic acid preparation and PCR-protocol, respectively. As diagnoses are time-sensitive to reduce the risk of viral transmissions and to avoid quarantine based staff shortage, fast turnover times are a mandatory requirement. As the pandemic has shown that both human and technical resources may quickly become limited, molecular diagnostic methods reducing hands-on time and technical effort while keeping high quality standards become highly important by extending already established diagnostic tests for future epidemic waves. Therefore, we performed a two stage pilot study with the novel in vitro diagnostics (IVD) labeled Takara SARS-CoV-2 Direct PCR detection assay in which RNA of heated specimen-containing pretreatment-mix is added directly into the real-time reverse transcription-PCR (rt-RT-PCR) reaction.

First, we analysed the technical sensitivity of the assay by using two reference samples named BP3 (BP = “Bezugs-Probe”) and BP4 obtained by INSTAND e.V. (INSTAND, Düsseldorf, Germany). These samples, adjusted to viral RNA loads of about 10.7 (BP3) and 10.6 (BP4) copies/ml, are based on cell culture supernatants derived from SARS-CoV-2 infected Vero-cells [1]. Dilution series were prepared in duplicate to determine the correlation between viral RNA load and crossing threshold (Ct)-value in the Takara assay (Fig. 1). The average N-gene Ct-value difference between the undiluted reference samples BP3 (Ct 24.54) and BP4 (Ct 27.87) is 3.33 compared to 3.44 ± 0.64 previously reported by INSTAND e.V. [1]. Copies of the N-gene could be detected down to 500 copies/ml sample taking into account that the measuring range of 10^2 – 10^3 copies/ml represents the detection limit in the given test system.

The next step was to retest throat wash specimens of hospital staff (n = 95), which were previously tested by the Altona Diagnostics RealStar SARS-CoV-2 RT-PCR (Altona Diagnostics, Hamburg, Germany) with the IVD labeled Takara SARS-CoV-2 Direct PCR detection assay. Regarding the Altona PCR 37.9% (n = 36) of the samples were negative and 62.1% (n = 59) were positive for SARS-CoV-2 with Ct-values ranging from 19.89/20.94 (S-/E-gene) to 37.26/37.77 (S-/E-gene) when processed with a COBAS z480 (Roche, Mannheim, Germany). In comparison the overall test sensitivity of the Takara assay was 91.53% (CI: 0.817-0.963) due to deviations regarding samples with low viral load. As no false positive samples were detected the overall specificity was 100% (CI: 0.904-1.0). According to the RKI (Robert Koch-Institut) recommendations [2] Ct-value 30 is set as the critical value to distinguish whether recovered or reconvalescent medical staff is allowed to resume work. For this reason we analysed an additional subcohort (n = 86) including 50 Altona-PCR positive samples with Ct-values < 35. This value was chosen, because analyses regarding the reference samples BP3 and BP4 revealed that N-gene Ct-values are about three cycles later than E-gene Ct-values [1]. The evaluation of the Takara test performance in this Ct-value range resulted in a sensitivity of 98.00% (CI: 0.895–0.997) with still 100% specificity.

While the use of Ct-value thresholds regarding risk-benefit ratio was already discussed [3] the Ct-value 30 is among others considered as a decision-making tool in Germany [2], although it was clearly demonstrated in round robin trials that Ct-values of single specimens may vary seriously among different labs using the same method and even more among different labs using different methods [4].

Leaving aside these issues, the present study show that below Ct-value 35 the Takara assay performs as good as the Altona
Real Star SARS-CoV-2 assay. Taking into account the fact that no further RNA-extraction is required, which improves overall processing time, the Takara assay is a real alternative without the need for further equipment. Currently, the company develops the second generation of the assay, which will comply with the European In Vitro Diagnostics Regulations (IVDR) guidelines entered into force in Europe in May 2022.

Transparency declaration

The authors declare no conflicts of interest.

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FIG. 1. Determination of technical sensitivity. Dilution series of the quantitative reference samples BP3 (10.7 copies/mL) and BP4 (10.6 copies/mL) were performed to correlate Ct-value and viral RNA load.