

Identification of the British variant (20I/501Y.V1) using the *COVID-19 Multiplex RT-PCR Kit* (Application note, DIANA Biotechnologies s.r.o.)

COVID-19 Multiplex RT-PCR Kit (Cat# DB-1211) is used for one-step RT-PCR detection of SARS-CoV-2 virus (Wuhan coronavirus, COVID-19) from extracted RNA. The kit specifically amplifies and detects viral **Spike** and **EndoRNase** genes in a single multiplexed RT-PCR reaction, along with **artificial RNA isolation control**. This control is added before the RNA isolation step and thus controls not only the efficacy of RT-PCR reaction but also of the RNA isolation process.

Targeted sequences of the COVID-19 virus did not contain frequent mutations by the end of 2020 except the mutation in Spike A570D. This mutation is present almost exclusively in the British variant (lineage B.1.1.7, GISAID 20I/501Y.V1, annotation VUI 202012/01). As of 15th January 2021, 17,751 sequences with A570D mutation were found in the GISAID database (out of total 373 229 sequences) and 17,388 (98%) of these sequences were annotated as VUI 202012/01 variant. The Spike is detected despite the mutation, but the British variant can be discriminated from other variants because of the shift of the Ct value by few cycles in HEX channel. Detection in FAM channel is not affected and the test sensitivity thus remains the same despite the mutation. All samples with the Ct difference between FAM and HEX channels fulfilling conditions described below contain with high probability the British variant. High number of samples with such shift in Ct values were identified using this kit during January 2021 in the Czech Republic. The Czech National Institute of Health selected and sequenced 36 of these samples and confirmed the British variant in all 36 cases. A guideline released by the *National Reference Laboratory for Influenza and non-Influenza Respiratory Virus Diseases* on 26th January 2021 states that it is not necessary to confirm by sequencing samples identified by this kit as the British variant.

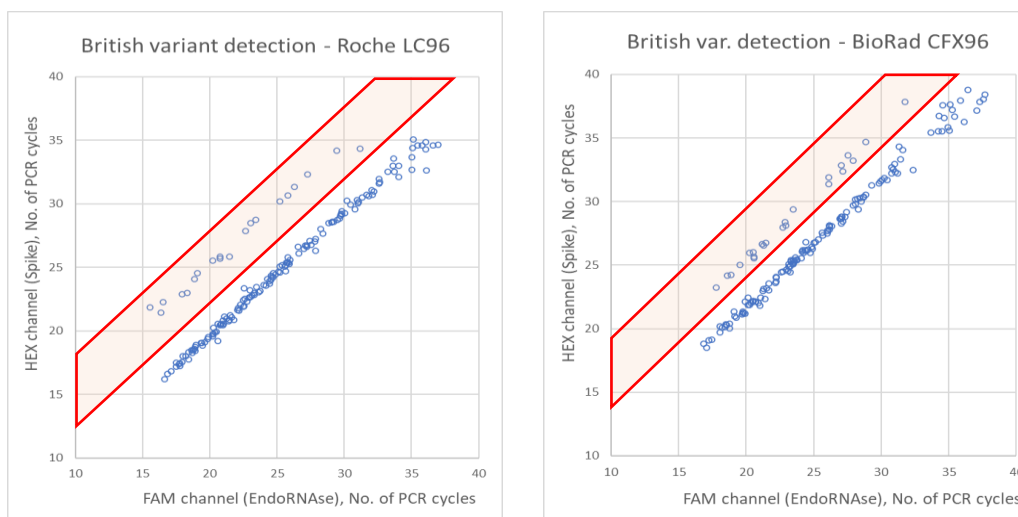


Figure 1: Detection of British variant by comparison of Ct values of FAM and HEX channels

Correlation of Ct-values in FAM and HEX channels in clinical samples. Data from Roche LC96 are displayed on the left chart and data from BioRad CFX96 are on the right chart. Samples within red area contain the British variant.

A typical difference of Ct values in FAM and HEX channels is less than one cycle on Roche LightCycler 96 and LightCycler 480 II. The difference on BioRad CFX96 is usually around one cycle. Ct value in HEX channel is typically 4-5 cycles higher for the British variant compared to other variants (i.e., a total shift in Ct values is between 5 and 6 cycles in HEX channel compared to FAM channel). To identify the British variant, first calculate the difference $Ct(\text{HEX}) - Ct(\text{FAM})$ for the positive control included in the kit. This difference is

called as the "**reference shift**". All samples with Ct(H_{HEX}) – Ct(F_{AM}) by two or more cycles higher than the reference shift contain with a high probability the British variant (i.e. the A570D mutation). The difference of 4-5 cycles compared to the reference shift is typical to above mentioned cyclers, but it can be even higher on other cyclers (e.g., Agilent AriaMx). **Figure 1** shows typical results on Roche LC96 and BioRad CFX96 cyclers. Absence of signal in HEX channel can appear in samples having Ct values in FAM channel higher than 35th cycle. Such samples can be stated as positive without repeated analysis.

DB-1229 COVID-19 Primer additive B.1.1.7

DIANA Biotechnologies also supplies an additive that minimizes shift in HEX channel in the British variant.

The additive contains primer concentrate for more efficient amplification of the British variant B.1.1.7. After adding this additive, the Ct value in HEX channel is approximately by 1.5 cycle higher in British variant over other variants, while it is 4-5 cycles higher using this kit without the additive. Shift by 1.5 cycle is still sufficient for identification of the British variant, but with a minimal change in the HEX assay sensitivity.

We recommend using this additive when the Ct shift in British variant is higher than 5 cycles on your cycler (e.g., Agilent AriaMx). However, it is not necessary to use the additive in combination with BioRad CFX96 cyclers since the sensitivity of detection in HEX channel is not significantly affected by the mutation: signal in HEX is still detectable in samples with a Ct value in FAM of up to 35th-36th cycle.

The use of **DB-1229 Primer additive (200x)** for increased amplification of the British variant is simple: just add Primer additive (11 µL in kit for 100 reactions, 110 µL in kit for 1000 reactions) into Primer mix (Vial No.2, blue ● or transparent cap) and mix thoroughly, before the first use of the DB-1211 kit. Use this mix instead of the original primer mix.

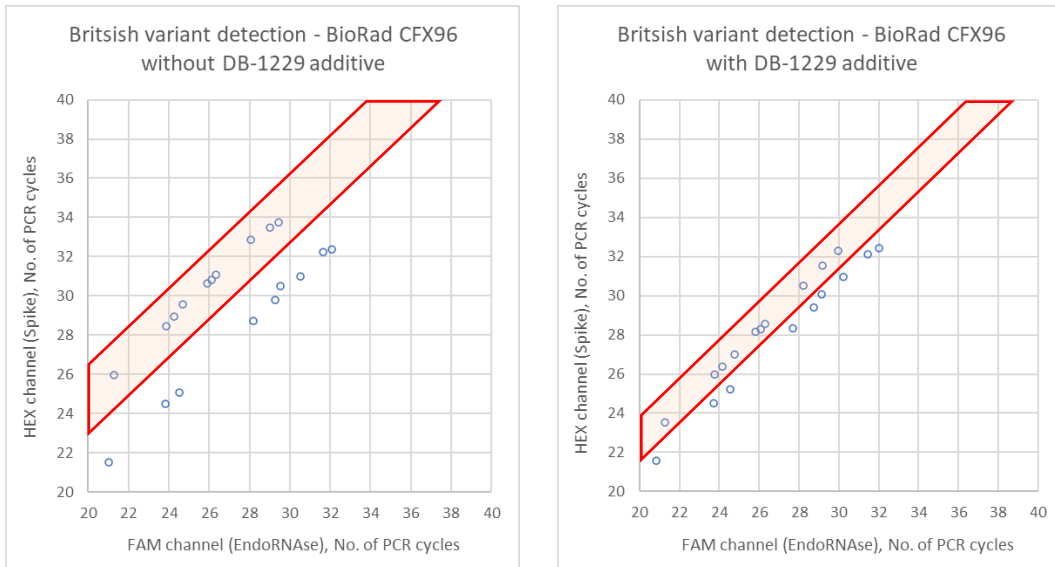


Figure 2: Detection of British variant by comparison of Ct values in FAM and HEX using DB-1229 additive. Correlation of Ct-values in FAM and HEX channels in clinical samples on BioRad CFX96 cycler. Results without the DB-1229 primer additive are shown on the left chart, whereas results with the DB-1229 primer additive are shown on the right chart. Samples within the red area contain the British variant.