**From Crisis to Routine - Standardization of SARS-CoV-2 Genome Detection by Enhanced EQA Schemes in a Scientific Pandemic Network**

M Kammel et al.

**Supplementary Tables incl. Legends**

**Supplementary Table 1: A listing of the samples, properties, dates, and results for the 13 EQA schemes**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| A | B | C | D | E | F | G | H |
| **Isolate/**  **Sample material** | **SARS-CoV-2 RNA** | **Variant of concern (VOC) / lineage** | **Type of inacti-vation** | **Sample No** | **EQA scheme date** | **Dilution of the SARS-CoV-2 RNA-positive material** | **SARS-CoV-2 RNA load in copies/mL**  (robust average of all reported quantitative results for each sample) |
| SARS-CoV-2, BetaCoV/Munich/ChVir984/2020, cell culture supernatant | positive | non-VOC /  B.1 | heat | 340059 | 2020 April | 1 : 1 000 | 17 071 604 |
| 340077 | 2020 November | 1 : 7 500 | 1 024 516 |
| 409005 | 2021 March | 1 : 7 500 | 1 045 777 |
| 340063 | 2020 April | 1 : 10 000 | 2 198 982 |
| 340069 | 2020 June | 1 : 50 000 | 268 471 |
| 409020 | 2021 November | 1 : 50 000 | 162 059 |
| 409021 | 2022 March | 1 : 50 000 | 187 065 |
| 340080 | 2020 November | 1 : 75 000 | 120 084 |
| 409013 | 2021 September | 1 : 75 000 | 166 281 |
| 340064 | 2020 April | 1 : 100 000 | 220 046 |
| 340073 | 2020 June | 1 : 500 000 | 25 002 |
| 409009 | 2021 June | 1 : 500 000 | 19 112 |
| 340061 | 2020 April | 1 : 1 000 000 | 25 978 |
| 340066 | 2020 June | 1 : 5 000 000 | 2 943 |
| 340071 | 2020 June | 1 : 50 000 000 | 452 |
| SARS-CoV-2, BetaCoV/Baden-Wuerttemberg/1/ChVir1577/2020 IsolatBER, cell culture supernatant | positive | non-VOC /  B.1 | heat | 340079 | 2020 November | 1 : 50 000 | 204 795 |
| 409-230314-02 | 2023 March | 1 : 50 000 | 351 642 |
| SARS-CoV-2, BetaCoV/Berlin/ChVir1670/2020 IsolatBER, cell culture supernatant | positive | non-VOC /  B.1 | heat | 340075 | 2020 November | 1 : 50 000 | 231 993 |
| 409-230613-05 | 2023 June | 1 : 167 500 | 95 996 |
| SARS-CoV-2, BetaCoV/Munich/BavPat9-ChVir1433/2020 IsolatBER, cell culture supernatant | positive | non-VOC /  B.1 | heat | 409001 | 2021 March | 1 : 1 000 | 16 359 084 |
| 409015 | 2021 September | 1 : 10 000 | 2 050 128 |
| 409011 | 2021 September | 1 : 100 000 | 264 641 |
| SARS-CoV-2, BetaCoV/Passau/ChVir21652/2020 VOC, cell culture supernatant | positive | Alpha / B.1.1.7 | heat | 409002 | 2021 March | 1 : 2 500 | 7 218 064 |
| 409006 | 2021 June | 1 : 25 000 | 673 794 |
| 409022 | 2022 March | 1 : 25 000 | 417 939 |
| 409036 | 2022 November | 1 : 25 000 | 556 654 |
| 409018 | 2021 November | 1 : 50 000 | 261 805 |
| 409-230613-04 | 2023 June | 1 : 2 500 000 | 11 479 |
| SARS-CoV-2, BetaCoV/South Africa/ChVir22131/2020, cell culture supernatant | positive | Beta / B.1.351 | heat | 409003 | 2021 March | 1 : 3 500 | 6 290 377 |
| 409008 | 2021 June | 1 : 35 000 | 681 366 |
| 409030 | 2022 June | 1 : 350 000 | 92 064 |
| 409-230314-01 | 2023 March | 1 : 350 000 | 117 581 |
| SARS-CoV-2, hCoV-19/Germany/SH-ChVir25702\_4/2021, cell culture supernatant | positive | Delta / B.1.617.2 | heat | 409014 | 2021 September | 1 : 19 000 | 877 374 |
| 409027 | 2022 June | 1 : 19 000 | 909 842 |
| SARS-CoV-2, hCoV-19/Germany/SH-ChVir25702\_4/2021, cell culture supernatant | positive | Delta / B.1.617.2 | chemi-cally | 409016 | 2021 November | 1 : 5 500 | 3 250 517 |
| 409012 | 2021 September | 1 : 11 000 | 879 635 |
| 409023 | 2022 March | 1 : 11 000 | 1 344 707 |
| 409031 | 2022 September | 1 : 11 000 | 1 503 872 |
| SARS-CoV-2, hCoV-19/Germany/SH-ChVir26373/2021, cell culture supernatant | positive | Omicron BA.1 / B.1.1.529 | heat | 409029 | 2022 June | 1 : 6 000 | 1 032 818 |
| 409025 | 2022 March | 1 : 60 000 | 125 931 |
| 409034 | 2022 September | 1 : 60 000 | 167 508 |
| SARS-CoV-2, v8\_26729\_2\_V.V.p7, cell culture supernatant | positive | Omicron BA.2 / B.1.1.529 | chemi-cally | 409-230314-04 | 2023 March | 1 : 20 000 | 1 543 258 |
| 409026 | 2022 June | 1 : 200 000 | 95 978 |
| 409035 | 2022 September | 1 : 200 000 | 170 113 |
| 409037 | 2022 November | 1 : 200 000 | 116 422 |
| 409033 | 2022 September | 1 : 2 000 000 | 11 615 |
| SARS-CoV-2, v46\_29061\_V.V.p5, cell culture supernatant | positive | Omicron BA.4 / B.1.1.529 | chemi-cally | 409038 | 2022 November | 1 : 1 500 000 | 34 267 |
| SARS-CoV-2, v45\_29057\_V.V.p5, cell culture supernatant | positive | Omicron BA.5 / B.1.1.529 | chemi-cally | 409-230613-02 | 2023 June | 1 : 35 360 | 666 977 |
| 409-230314-03 | 2023 March | 1 : 353 600 | 113 714 |
| 409040 | 2022 November | 1 : 480 000 | 59 172 |
| SARS-CoV-2, V80\_41245\_V.V.p4, cell culture supernatant | positive | Omicron XBB.1.5 / B.1.1.529 | chemi-cally | 409-230613-01 | 2023 June | 1 : 250 000 | 80 360 |
| in vitro transcripts | positive | not applicable | heat | 340081 | 2020 November | 1 : 10 000 | not applicable |
| Beta coronavirus OC43, cell culture supernatant | negative | - | - | 340074 | 2020 June | 1 : 1 000 | 0 |
| 340060 | 2020 April | 1 : 2 500 | 0 |
| Beta coronavirus MERS-CoV, cell culture supernatant | negative | - | heat | 340067 | 2020 June | 1 : 1 000 | 0 |
| Alpha coronavirus NL63, cell culture supernatant | negative | - | - | 340072 | 2020 June | 1 : 1 000 | 0 |
| 409010 | 2021 June | 1 : 1 000 | 0 |
| Alpha coronavirus 229E, cell culture supernatant | negative | - | - | 340068 | 2020 June | 1 : 1 000 | 0 |
| 340078 | 2020 November | 1 : 1 000 | 0 |
| 409017 | 2021 November | 1 . 2 000 | 0 |
| 340065 | 2020 April | 1 : 2 500 | 0 |
| MRC-5, cell lysate | negative | - | - | 409007 | 2021 June | - | 0 |
| 340062 | 2020 April | - | 0 |
| 340070 | 2020 June | - | 0 |
| 409004 | 2021 March | - | 0 |
| 340076 | 2020 November | - | 0 |
| 409019 | 2021 November | - | 0 |
| 409024 | 2022 March | - | 0 |
| 409032 | 2022 September | - | 0 |
| 409028 | 2022 June | - | 0 |
| 409039 | 2022 November | - | 0 |
| 409-230314-05 | 2023 March | - | 0 |
| 409-230613-03 | 2023 June | - | 0 |

**Legend to Supplementary Table 1**

The table shows all EQA samples used and their properties for the 13 EQA schemes for SARS-CoV-2 genome detection for the period April 2020 to June 2023.

The SARS-CoV-2 RNA-positive isolates or variants used, the in vitro transcript of SARS-CoV-2 RNA, the control materials that were positive for MERS coronavirus or positive for human coronaviruses, as well as non-infected cell culture lysates are shown in **column A**. Further information is given on the characteristics of the samples (positive or negative for SARS-CoV-2 RNA) (**column B)**, the SARS-CoV-2 variant used (**column C)**, the type of inactivation (**column D)**, the sample number in the corresponding EQA scheme (**column E)**, the date of the EQA scheme (**column F)**, the dilution of the SARS-CoV-2 RNA-positive material (**column G)** and the respective SARS-CoV-2 RNA load, calculated as a robust average of all reported quantitative results (in copies/mL) for each of the samples (**column H)**.

All reports are available upon request at instand@instand-ev.de.

**Supplementary Table 2:** **INSTAND EQA Scheme (340) for Virus Genome Detection of SARS-CoV-2 - June 2020 – qualitative results and *“Overall Ct/Cq medians”***

|  |  |  |  |
| --- | --- | --- | --- |
| A | B | C | D |
| **Sample no.** | **Dilutions of SARS-CoV-2-positive samples** | **Success rates of correct positive results (%)**  (for all target gene regions and all test systems)  [number of  correct results per  total number  of reported results] | ***“Overall Ct/Cq medians”***  for all target gene regions and all test systems  according to category 1  for Ct/Cq value consideration |
| **340069** | 1 : 50 000 | **98.3%**  [1 460/1 486] | **28.2** |
| **340073** | 1 : 500 000 | **96.5%**  [1 434/1 486] | **31.5** |
| **340066**  Revealed sample | 1 : 5 000 000 | **Not evaluated** (90.2%)  [1 341/1 486] | **34.4** |
| **340071**  Challenge sample | 1 : 50 000 000 | **Not evaluated** (59.9%)  [890/1 486] | **36.8** |

**Legend to Supplementary Table 2**

Supplementary Table 2 comprises for each of the four SARS-CoV-2-positive EQA samples of the second EQA scheme June 2020 the sample numbers (**column A**), the dilutions of the SARS-CoV-2-positive material (**column B)**, the success rates of correct positive results (number of correct positive results per reported results) (**column C**) as well as the *"overall Ct/Cq medians"* (generated by including the results for all target gene regions and all test systems; according to category 1 for Ct/Cq value consideration; see section 3.1.2) (**column D**). The positive samples no. 340059, 340061, 340063 and 340064 represent consecutive dilution steps of a dilution series of one and the same supernatants of cells which were infected with SAR-CoV-2 virus. See also Report for EQA scheme April 2020 virus genome detection of SARS-CoV-2 (Zeichhardt and Kammel, 2020c).

**Supplementary Table 3: Example for variation of Ct/Cq values reported in the EQA scheme June 2023 – Ct/Cq values for sample no.409-230613-01 differentiated by target gene regions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| A | B | C | D | E | F |
| **Target gene region** | ***“Target gene-specific Ct/Cq median"***  (for all test systems)  according to category 2 for Ct/Cq value consideration | **Minimum value for the**  **"*assay-specific Ct/Cq median*"**  (for the specific gene region)  according to  category 3  for Ct/Cq value consideration | **Maximum value for the**  **"*assay-specific Ct/Cq median*"**  (for the specific gene region)  according to  category 3  for Ct/Cq value consideration | **Difference value between**  **D and C**  (for the specific target gene region) | **Total number of**  **analyses** |
| **N gene** | 29.9 | 23.2 | 36.2 | 13.0 | 107 |
| **E gene** | 30.0 | 27.0 | 35.8 | 8.8 | 129 |
| **S gene** | 28.5 | 26.1 | 31.6 | 5.5 | 22 |
| **RdRP gene** | 30.1 | 12.1 | 36.2 | 24.1 | 69 |
| **ORF 1a** | 31.2 | 29.8 | 33.6 | 3.8 | 9 |
| **ORF 1ab** | 29.7 | 27.0 | 32.7 | 5.7 | 60 |
| **not specified by participants** | 29.7 | 24.8 | 36.4 | 11.6 | 8 |

**Legend to Supplementary Table 3**

Overview for the different Ct/Cq medians for sample no.409-230613-01 (SARS-CoV-2 RNA-positive, diluted 1 : 250 000) of the EQA scheme June 2023 differentiated by the reported results for each target gene region.

For this sample the *“overall Ct/Cq median”* was 29.9 (generated by including the results for all target gene regions and all test systems; category 1 for Ct/Cq value consideration; see section 3.1.2) (see Supplementary Figure 1). Shown are the different target gene regions (**column A**), the *“target gene-specific Ct/Cq medians”* (generated for the specific target gene region from the reported results of all applied test systems; category 2 for Ct/Cq value consideration) (**column B**), the corresponding minimum and maximum *"assay-specific Ct/Cq medians”* (generated for a defined test system from the reported results for a specific target gene region; category 3 for Ct/Cq value consideration) (**columns C and D**), the difference values between the minimum and maximum *"assay-specific Ct/Cq medians"* described in columns C and D (**column E**) and the number of analyses for each of the target gene regions (**column F**) (Zeichhardt and Kammel, 2023a).

It must be taken into account that the extreme results of the submitted Ct/Cq values may be influenced by strongly deviating incorrect analyses and/or reporting errors.