ANCHOR

SARS-CoV-2 PCR Kit v 2.0

Instructions for Use

Anchor SARS-CoV-2 PCR Kit v 2.0

CE

Qualitative Real-Time RT-PCR Kit

for *in vitro* diagnostic use

IVD For *in vitro* diagnostic use

REF A2100

Σ 384

HB A2110-UK – 25.05.2022

QG A2111-UK – 25.05.2022

-30°C to -15°C

ANCHOR Diagnostics GmbH
Grandweg 64
D-22529 Hamburg

compatible with

LightCycler 480 II (Roche)

cobas z 480 (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

QuantStudio 5 (Applied Biosystems)

Mic qPCR (Biomolecular Systems)

1 Contents

2 Intended Use

The Anchor SARS-CoV-2 PCR Kit v 2.0 is an *in vitro* nucleic acid amplification test based on Real Time PCR technology for the qualitative detection of human SARS coronavirus 2 RNA, isolated from human respiratory swabs, bronchoalveolar lavage (BAL) specimen, sputum, and tracheal aspirates.

The product is intended to be used by professional operators, such as technicians and physicians who are trained in molecular biological techniques.

3 Product Description

The Kit constitutes a ready-to-use system for the reverse transcription, amplification and detection of SARS-CoV-2 specific S- and N-gene segments.
In addition, a heterologous amplification system (Internal Control) is included to supervise the success of the sample extraction procedure and to identify possible inhibition of the amplification reaction.
Probes linked to distinguishable fluorescent dyes enable the parallel detection of SARS-CoV-2 specific nucleic acids and the Internal Control in two corresponding detector channels of the Real Time PCR instrument.
The Positive Control SARS-CoV-2 contains a defined concentration of synthetic RNA bearing the SARS-CoV-2 target sequences. It is used together with the Negative Control RNA 2 to monitor the integrity of the analyte-specific reagents of the kit and the proper performance of the reaction.

4 Kit Components

Master A and Master B reagents contain all necessary components to allow RT-PCR mediated reverse transcription, amplification and target detection of SARS-CoV-2 specific RNA and Internal Control in one reaction setup. The Positive Control SARS-CoV-2 and NC (Negative Control) RNA 2 are supplied with the IC (Internal Control) RNA 2 already incorporated (see also section 9.2.1 Master Mix Set-Up).
The reagents provided with the kit allow the preparation of 384 reactions.

Master A SARS-CoV-2	Master B SARS-CoV-2	IC RNA 2	! PC SARS-CoV-2	! NC RNA 2
A2101	A2102	A0023	A1603	A0033
4 Vials	4 Vials	4 Vials	1 Vial	1 Vial
4x 480 µL	4x 480µL	4x 1000 µL	200 µL	200 µL
Contains: Buffer, Bovine Serum Albumin, Polymerase, Reverse Transcriptase	Contains: Buffer, Salt, Nucleotides, Target- and IC-specific Oligonucleo-tides	Contains: Buffer, IC-specific synthetic Polynucleo-tide	Contains: Buffer substance, Target-specific synthetic Polynucleotide	Contains: Buffer substance, IC-specific synthetic Polynucleo-tide

! INTERNAL CONTROL INSIDE !

5 Storage and Stability

- The Anchor SARS-CoV-2 PCR Kit v 2.0 is shipped on dry ice and should be stored at –30 to –15°C upon receipt.
- The components are stable until the expiration date stated on the label.
- Do not use components of the kit that have passed their expiration date.
- Store SARS-CoV-2 RNA-positive and/or potentially positive material separated from the kit.
- Repeated thawing and freezing of the Master reagents of > 3x should be avoided, as this may reduce the assay performance. For the PC SARS-CoV-2, the NC RNA 2 and the IC RNA 2, thawing and freezing cycles up to 4x are allowed. Alternatively, storage between +2 to +8°C for up to 14 days is possible.
- Due to the components used it might be possible that Master vials do not always freeze completely after initial thawing. This is not a matter of concern and does not influence the stability or performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Label aliquots clear and unambiguously to avoid a mix-up of reagents.
- During PCR set up the reagents should be kept cooled at +2 to +8°C – use a cooling block.
- Do not store Master A and Master B SARS-CoV-2 more than 3 h at +2 to +8°C.
- Protect all reagents from extensive light exposure.

6 Material Required but Not Provided

- Nucleic acid purification system
- Real Time PCR instrument
- Appropriate PCR reaction vessels and related accessories
- Cooling block (for reaction setup)
- Benchtop centrifuge (rotor holding 2 mL reaction tubes)
- Vortex mixer
- Pipettes (variable volume)
- Single-use pipette filter tips
- 1.5 mL or 2 mL reaction tubes (for Master mix set-up)
- Single-use gloves (powder-free)

Use all materials and equipment according to the manufacturer's instructions. Maintain and calibrate the equipment as recommended.

7 Limitations

- Strict compliance with the user manual is required for optimal PCR results.
- Any diagnostic results generated must be interpreted in conjunction with other clinical and/or laboratory findings.
- The presence of PCR inhibitors may cause invalid results.
- Occurrence of mutations within the target region might result into a reduced sensitivity or a complete detection failure.
- Following good laboratory practices is crucial for the successful usage of the product.
- Appropriate handling of the reagents is essential to avoid contaminations or impurities.

8 Warnings and Precautions

- For *in vitro* diagnostic use.
- Use of this product is limited to personnel specially instructed and trained in the techniques of Real Time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as potentially infectious and/or as biohazardous material in accordance with safe laboratory procedures.
- The Anchor Master A SARS-CoV-2 contains a bovine sourced potentially infectious component (albumin). The bovine plasma is sourced from New Zealand or USA, which are recognized by the world organization for animal health *Office International des Epizooties* (OIE, Paris) as having a negligible BSE risk.
- Wear protective single-use gloves, a laboratory coat and eye protection when handling specimens or kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free single-use pipette tips with aerosol barriers.
- Use separated working areas for (1) specimen preparation, (2) PCR reaction set-up and (3) amplification/detection activities.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Discard sample and assay waste according to your local safety regulations.

9 Workflow

9.1 Sample Preparation

9.1.1 Sample Mix

The recommended patient sample matrices for sample preparation input are:

- human respiratory swabs
- bronchoalveolar lavage specimen (BAL)
- sputum
- tracheal aspirate fluids

For swab samples only materials specified for the detection of viral targets should be used. Sample taking, handling and shipment procedures should follow the WHO recommendations specified in the guidance document "Laboratory biosafety guidance related to coronavirus disease (COVID-19)"[1]. If a sample is not directly processed, store samples according to manufacturer's instructions (Collection Tube, Transport Tube, Sample Preparation Kit) before use.
Storage recommendations according to „RKI – Considerations for testing of patients for infections with the novel coronavirus SARS-CoV-2[2]:

All samples should be processed as soon as possible. Samples could be stored at 4 degrees Celsius for up to 72 hours.

[1] Version 13 May 2020
[2] Version 12 March 2021

9.1.2 Sample Preparation

Purified RNA is the sample input material for the Anchor SARS-CoV-2 PCR Kit v 2.0. It has to be ensured that the chosen nucleic acid purification method is compatible with Real-Time PCR technology. The extraction has to be executed according to the manufacturer's instructions.

The diagnostic applicability of the Anchor SARS-CoV-2 PCR Kit v 2.0 has been shown using the following sample preparation systems:

Sample Preparation systems
NucliSENS® easyMag® System (bioMérieux)
EMAG® (bioMérieux)
EZ1 Advanced XL / EZ2 Connect (QIAGEN)
QIAcube Connect (QIAGEN)
QIAsymphony® SP (QIAGEN)
MagNA Pure 96 System (Roche)
MagNA Pure Compact (Roche)
Maxwell® 16 / RSC Instruments (Promega)
KingFisher Systems (Thermo Fisher Scientific)
SEEPREP32™ (Seegene)
GenoXtract® (Hain Lifescience)
Maelstrom 9600 (TAN Bead)
EchoLUTION Viral RNA/DNA Swab 96 Kit (BioEcho)

ⓘ If sample eluates are not directly used for PCR analysis, store eluates at -30 to -15 °C. In case of using eluates repeatedly, avoid frequent thaw/freeze cycles (no more than 3 cycles).

ⓘ Eluates should be labelled clearly and unambiguously to avoid a mix-up of samples.

9.1.3 Internal Control

The Internal Control RNA 2 provided with the Anchor SARS-CoV-2 PCR Kit v 2.0 should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality.

ⓘ The Internal Control RNA 2 MUST NOT be added directly to the sample.

Always add the Internal Control RNA 2 after lysis buffer has been added to the sample.
The required volume of Internal Control RNA 2 per sample purification is defined by the chosen elution buffer volume.
Ten percent of the elution buffer volume used should be added to the sample/lysis mixture.

Examples:

- Elution buffer per sample: 200 µL -> IC RNA 2 volume: 20 µL
- Elution buffer per sample: 60 µL -> IC RNA 2 volume: 6 µL

ⓘ Secure the elimination of residual ethanol before elution of nucleic acids. Ethanol may inhibit the amplification process.

If no co-purification of the Internal Control is planned and the IC RNA 2 should be used only as an inhibition control of the reaction, either the amount of IC related to the used elution volume could be added to each eluate or 1.5 µL of the IC RNA 2 / per reaction should be added to the master mix (see section 9.2.1 Master Mix Set-Up).

9.2 PCR Preparation

9.2.1 Master Mix Set-Up

ⓘ Consider configuring the run settings of the PCR cycler software to have the instrument ready before starting the PCR reaction preparation (Refer to section 9.3 PCR Cycler Configuration.)

Prepare the Master Mix step by step:

- Thoroughly thaw Master components A and B.
- Mix Master A and B by gentle pipetting or short pulse-vortexing.
- Spin Master A and B shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- According to your preferred workflow follow one of the pipette schemes below to mix Master A and B using a 1.5 mL or 2 mL reaction tube:

IC RNA 2 present in sample eluates – NO IC RNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A SARS-CoV-2	5 µL (X)	55 µL	Y µL
Master B SARS-CoV-2	5 µL (X)	55 µL	Y µL
Volume Master Mix	10 µL	110 µL	Z µL

*10 reactions + 10%
** See formula on the next page

IC RNA 2 to be used as inhibition control only – IC RNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A SARS-CoV-2	5 µL (X)	55 µL	Y µL
Master B SARS-CoV-2	5 µL (X)	55 µL	Y µL
IC RNA 2	1.5 µL (X)	16.5 µL	Y µL
Volume Master Mix	11.5 µL	126.5 µL	Z µL

*10 reactions + 10%
** See formula on the next page

We recommend calculating for an additional volume of at least 10% to compensate potential loss during pipetting. The needed volume will be calculated by using the following formula:

** $N \times X \mu L x 1{,}1 = Y$

N = Number of reactions
X = Volume of component per reaction
Y = Total volume of component
Z = Total volume of Master Mix

- Mix prepared Master Mix well by gentle and short pulse-vortexing.
- Spin Master Mix shortly with a benchtop centrifuge to remove residual droplets from tube lids.

It is recommended to test the Positive Control and the Negative Control at least once in each PCR run.

The Positive Control SARS-CoV-2 and the Negative Control RNA 2 already contain the IC RNA 2 in a ready-to-use concentration.
No addition of IC necessary!

If you want to use a Master Mix preparation with added IC RNA 2 (as inhibition control) in combination with the Positive Control SARS-CoV-2 and NC RNA 2 , be aware that the IC signal of the controls will slightly shift towards a lower CT value in comparison to the IC signal of the controls using a Master mix without additional IC.

9.2.2 PCR Reaction Set-Up

ⓘ Always use a cooling block for the preparation of the PCR reaction mix.

Prepare the Reaction Mix step by step:

- If previously stored frozen, thaw eluates containing nucleic acid (and IC RNA 2) thoroughly.
- Mix eluates by gentle pipetting or brief pulse-vortexing.
- Spin eluates shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- Pipette **10 µL of Master Mix** (see section 9.2.1 Master Mix Set-Up) into suitable reaction vessels for PCR analysis. This is also valid for Master Mix spiked with IC RNA 2.
- Add **15 µL of eluate** or control (Positive Control SARS-CoV-2 or Negative Control RNA 2). **Mix well by repeated up and down pipetting.**
- Close reaction vessels securely with the appropriate sealing system.
- Immediately transfer closed and ready-to-use reaction vessels to the Real Time PCR instrument. Avoid any delays!

ⓘ Carefully handle reaction vessels during transfer to avoid mix up of samples.

ⓘ **Complete mixing of Master Mix reagents with a sample or control during reaction set up should be unconditionally secured by repeated up and down pipetting!**
This is essential to achieve an optimum amplification curve performance!!!

Master Mix	+	Eluate / Control	=	Reaction Mix
10 µL		15 µL		25 µL

9.3 PCR Cycler Configuration

The Anchor SARS-CoV-2 PCR Kit v 2.0 has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 30 min.
Cobas z 480 (Roche)	≈ 32 min.
LightCycler 480 II (Roche)	≈ 32 min.
CFX96 (Bio-Rad)	≈ 35 min.
Rotor-Gene Q (QIAGEN)	≈ 42 min.
Mic qPCR (BMS)	≈ 35 min.

The listed run times for the different instruments are effectively measured durations and can differ from what is displayed on the graphical user interface of the individual instrument software. For basic information concerning set-up and programming of the respective Real Time PCR instrument, refer to the instrument-specific manual.

9.3.1 Temperature Profile

Temperature cycling profile for **QuantStudio 5, Cobas z 480, LightCycler 480 II, CFX96 and Rotor-Gene Q**:

Hold	50°C	120 sec	x 1
Cycling	95°C	1 sec	x 40
	65°C *	2 sec	
	72°C	1 sec	

*Fluorescence acquisition for SARS-CoV-2 and IC

Temperature cycling profile for **Mic qPCR**:

Hold	50°C	120 sec	x 1
Cycling	95°C	1 sec	x 40
	63°C *	2 sec	
	72°C	1 sec	

*Fluorescence acquisition for SARS-CoV-2 and IC

Reaction Volume: 25 µL

9.3.2 Specific PCR Cycler Settings

The following table contains PCR cycle-specific recommendations for the basic configuration of the run settings. For additional information regarding the cycler settings recommended plastics, colour compensation, gain optimisation settings, etc. do not hesitate to contact us directly (see section 12 Technical Assistance & Contact Information)

Instrument	Target	Detection channel	Recommendations / Requirements
LightCycler® 480 II (Cobas z 480)	SARS-CoV-2	465/510	**Run Settings:** ▪ Block size: 96 ▪ If clear plates are used, the sensor of the LightCycler® has to be disabled by selecting the Clear Plates option in the software before the run is started. **Consumables:** ▪ LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001) ▪ LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001 ▪ LC480 Sealing Foil (Roche Mat. No. 04729757001)
	IC	533/580 (540/580)	
Bio-Rad CFX96	SARS-CoV-2	FAM	**Consumables:** ▪ Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655) ▪ Optical flat 8 Cap Strip for 0.2ml (Mat. No. TCS0803) ▪ 0.2 ml 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad Mat. No. TLS 0851) ▪ 8 strip optical clear flat caps (Sarstedt Mat. No. 65.1998.400)
	IC	HEX	
Rotor-Gene Q	SARS-CoV-2	Green	**Run Settings:** ▪ Use 72-Well Rotor ▪ Perform Auto-Gain optimisation before 1 st acquisition. **Consumables:** ▪ Strip Tubes and Caps, 0.1 ml (QIAGEN Mat. No 981103)
	IC	Yellow	

Instrument	Target	Detection channel	Recommendations / Requirements
Quant Studio™ 5	SARS-CoV-2	FAM	**Run Settings:** ▪ Block Type: 96 Well 0. 1 mL Block ▪ Experiment Type: Standard Curve Chemistry: TaqMan® Reagents ▪ Run Mode: Fast ▪ Plate attributes: Passive Reference None **Consumables:** ▪ 96 Well Fast Thermal Cycling Plates (Life Technologies Mat.No. 4346907) ▪ MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971)
	IC	HEX	
Mic qPCR	SARS-CoV-2	Green	**Run Settings** ▪ Temperature Control: Standard TAQ **Consumables:** ▪ Mic Tubes and Caps (Mat. No. 68MIC-60653)
	IC	Yellow	

9.4 Data Analysis

The following table contains cycler-specific references for the configuration of analysis settings. They could serve as an initial orientation. Depending on local cycler- and workflow-related differences adaptations might be necessary. For additional information concerning data analysis, refer to the instrument-specific manual of the respective Real Time PCR instrument or contact us (see section 12 Technical Assistance & Contact Information).

Instrument	Recommendations[1]
LightCycler® 480 II (Cobas z 480)	**Analysis Settings:** ▪ Abs Quant/Fit Points ▪ Color Comp (off) ▪ Mean ▪ High Confidence ▪ Threshold: - 465/510: 3.5 - 533/580: 3.8
Bio-Rad CFX96	**Analysis Settings (all channels):** ▪ Baseline Substracted Curve Fit ▪ C(t) Determination Mode: Single Threshold ▪ Baseline Threshold: - Baseline Cycles: Auto Calculated - Single Threshold - FAM: 2,000 - HEX: 1,500
Rotor-Gene Q	**Analysis Settings (all channels):** ▪ Quantitation ▪ Linear Scale ▪ Dynamic Tube ON ▪ Threshold: - Green: 0.08 - Yellow: 0.08
QuantStudio™ 5	**Analysis Settings (all channels):** ▪ Plot Type: ΔRn vs Cycle ▪ Graph Type: Linear ▪ Baseline Start/End: 3/15 ▪ Threshold: - FAM: 170,000 - HEX: 30,000
Mic qPCR	**Analysis Settings (all channels):** ▪ Graph Type: Linear ▪ Method: Dynamic ▪ Ignore Cycles Before: 3 ▪ Threshold Start: 1 ▪ Exclusion: None ▪ Threshold: - Green: 1.0 - Yellow: 1.4

9.4.1 Qualitative Analysis

For a valid run and as a prerequisite for the interpretation of the individual sample results, the following requirements have to be met by the included kit controls:

Channel/Target	SARS-CoV-2	IC
PC SARS-CoV-2	+	+
NC RNA 2	-	+

If one of the conditions has failed, result interpretation of the sample results might be flawed. In case of kit control failure, it is recommended to repeat the PCR run.

In case of a valid run, the following result interpretation can be made:

Qual. result	SARS-CoV-2	IC
SARS-CoV-2 RNA positive	+	+/-
SARS-CoV-2 RNA negative	-	+
Invalid	-	-

A positive result for SARS-CoV-2 RNA does not necessarily require a positive signal for the IC since high concentrations of the respective target nucleic acid can result in a competitive inhibition of the IC amplification.
An invalid result for a sample can be due to PCR inhibition or a failure during the nucleic acid isolation procedure. In such cases, it is recommended to dilute the nucleic acid extract 1:10 (recommended to be done in elution buffer, if possible) for a PCR retest or to repeat the nucleic acid isolation procedure. Note that the dilution of the nucleic acid extract might also lead to a reduction of the target nucleic acid concentration below the limit of detection of the Anchor SARS-CoV-2 PCR Kit v 2.0.

10 Performance Data

10.1 Analytical Performance

10.1.1 Sensitivity

The LOD for the Anchor SARS-CoV-2 PCR Kit v 2.0 was determined by undertaking a probit analysis on the Rotor-Gene Q platform. A dilution series of different concentration levels for SARS-CoV-2 RNA (Vircell, Mat # MBC137-R) was used. Each dilution level was tested with overall 24 replicates using 3 different PCR reagent lots across 2 different days, executed by 2 different persons on 2 different instruments.
The LOD value determined on the Rotor-Gene was then confirmed or re-evaluated on the other 4 instruments.

Instrument	LOD	Unit
Rotor-Gene Q	0.29	copies/µl
QuantStudio 5	0.58	copies/µl
LightCycler 480 II	0.58	copies/µl
CFX96	0.58	copies/µl
Mic qPCR	0.58	copies/µl

10.1.2 Specificity

Triplicates of eleven different SARS-CoV-2 isolates (whole genome synthesized or viral RNA) were tested at a concentration near the 3x LOD of the Anchor SARS-CoV-2 PCR Kit v 2.0.

Isolate	SARS-CoV-2
SARS-CoV-2/human/Australia/VIC01/2020	+
SARS-CoV-2/human/Wuhan-Hu-1	+
SARS-CoV-2/human/Japan/Hu_DP_Kng_19-020/2020	+
SARS-CoV-2/human/USA/TX1/2020	+
SARS-CoV-2/human/USA/MN2-MDH2/2020	+
SARS-CoV-2/human/USA/CA9/2020	+
SARS-CoV-2/human/DEU/HH-1/2020	+
SARS-CoV-2/human/Belgium/ULG-10004/2020 (B1.1.7)	+
SARS-CoV-2/human/England/205041766/2020 (B.1.1.7)	+
SARS-CoV-2/human/England/MILK-9E05B3/2020 (B.1.1.7)	+
SARS-CoV-2 Lineage B.1.351	+

Nucleic acid of selected pathogens with a concentration of ≈5.00E+03 copies/µL (alternative units CFU/µl or TCID50/µL) was added to the PCR reaction and tested in triplicates in the absence or presence of SARS-CoV-2 RNA at its 3x LOD concentration on the Rotor-Gene Q.

Pathogen	SARS-CoV-2	3x LOD SARS-CoV-2
Human bocavirus 1	-	+
Mumps virus	-	+
Measles virus	-	+
Adenovirus 2	-	+
Adenovirus 4	-	+
Adenovirus 5	-	+
Betacoronavirus 1 (OC43)	-	+
Human coronavirus 1 (229E)	-	+
Human coronavirus NL63	-	+
SARS coronavirus (2003)	-	+
MERS coronavirus 2c EMC/2012	-	+
Human enterovirus 68	-	+
Human enterovirus 71	-	+
Human enterovirus 68	-	+
Human enterovirus 71	-	+
Human parainfluenza virus 1	-	+
Human parainfluenza virus 2	-	+
Human parainfluenza virus 3	-	+
Human parainfluenza virus 4	-	+
Human respiratory syncytial virus A	-	+
Human respiratory syncytial virus B	-	+
Human rhinovirus 6	-	+

Pathogen	SARS-CoV-2	3x LOD SARS-CoV-2
Human rhinovirus 89	-	+
Influenza A virus (H1N1)	-	+
Influenza A virus (H3N2)	-	+
Influenza B virus (Yamagata)	-	+
Metapneumovirus	-	+
Mycoplasma pneumoniae	-	+
Streptococcus pneumoniae	-	+
Bordetella pertussis	-	+
Candida albicans	-	+
Chlamydophila pneumoniae	-	+
Haemophilus influenzae	-	+
Legionella pneumophila	-	+

10.1.3 Precision

Precision testing was initially performed on the Rotor-Gene Q instrument. For intra-run variability, 6 replicates of each sample dilution were tested within one run using one instrument and reagent lot by one operator. For inter-run variability, 6 replicates of each sample dilution were tested within overall four runs using two instruments and one reagent lot by two operators across days. For inter-batch variability, 6 replicates of each sample dilution were tested within one run using one instrument and three reagent lots by one operator.

PC SARS-CoV-2			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	25.76	0.07	0.29
Inter-Run	25.59	0.21	0.81
Inter-Batch	25.59	0.27	1.04
Total	25.64	0.20	0.78
SARS-CoV-2 RNA (PC concentration)			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	25.62	0.06	0.25
Inter-Run	25.81	0.13	0.49
Inter-Batch	25.86	0.19	0.73
Total	25.86	0.14	0.54
SARS-CoV-2 RNA (5x LOD)			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	33.93	0.43	1.28
Inter-Run	33.78	0.32	0.94
Inter-Batch	33.69	0.32	0.96
Total	33.78	0.31	0.91

Precision of the Anchor SARS-CoV-2 PCR Kit v 2.0 in combination with the other instruments was evaluated for intra- and inter-run variability.

10.2 Clinical Performance

The clinical performance of the Anchor SARS-CoV-2 PCR Kit v 2.0 for the qualitative detection of SARS-CoV-2 RNA in human respiratory swab samples was evaluated comparatively against a reference SARS-CoV-2 diagnostic workflow.
131 retrospectively collected specimen were analysed with the Anchor SARS-CoV-2 PCR Kit v 2.0 and with the comparator assays to determine their positive percent agreement (PPA) and negative percent agreement (NPA), respectively. Testing was done using the EZ1 Advanced XL sample preparation system in combination with the CFX96 (Bio-Rad) PCR cycler.

		Comparator	
	∑ 131	POS	NEG
Anchor SARS-CoV-2 PCR Kit v 2.0	POS	48	0
	NEG	2	81

PPA: 96.0 % NPA: 100.0 %

11 Quality Control

In accordance with the implemented ISO 13485-certified Quality Management System, each lot of the Anchor SARS-CoV-2 PCR Kit v 2.0 is tested against predetermined specifications to ensure consistent product quality.

12 Technical Assistance & Contact Information

For any questions, a need for technical assistance or if you identify difficulties using our products do not hesitate to contact us:

phone: +49 40 520 14 830
email: support@anchor-diagnostics.com

13 Literature

(1) https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19)

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(4) Corman et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan 23; 25(3): 2000045.

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(6) Zhai et al., The epidemiology, diagnosis and treatment of COVID-19. Int J Antimicrob Agents. 2020 Mar 28: 105955.

(7) Loeffelholz et Tang, Laboratory diagnosis of emerging human coronavirus infections – the state of the art. Emerging Microbes & Infections, 9:1, 747-756.

(8) Wang et al., The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur J Clin Microbiol Infect Dis. 2020 Apr 24: 1–7.

(9) Chen et al., Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol. 2020; 92:418-423.

(10) Khailany et al., Genomic characterization of a novel SARS-CoV-2. Gene Rep. 2020 Jun; 19: 100682.

14 Symbols

IVD For *in vitro* diagnostic use

REF Product - Catalogue number

Contains sufficient reagents for <N> tests

H B Instructions for Use - Catalogue number and version

Consult Instructions for Use

QG Quick Guide - Catalogue number and version

Temperature limits for storage

Use by

LOT Batch code

Important Note

Manufacturer

Notes

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