SARS-CoV-2 RT-qPCR DETECTION KIT

Qualitative detection of SARS-CoV-2 RNA by real-time multiplex fluorescent RT-qPCR

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CE IVD

INTENDED USE: IN VITRO DIAGNOSIS

205 rue des frères Lumière
69 970 CHAPONNAY
FRANCE
+33 (0)4 37 57 00 54
contact@appolonbioteck.com
www.appolonbioteck.fr

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I. Product name

SARS-CoV-2 RT-qPCR DETECTION KIT.

Qualitative detection of SARS-CoV-2 RNA by real-time multiplex fluorescent RT-qPCR

II. Intended use

SARS-CoV-2 RT-qPCR DETECTION KIT is CE-IVD marked according to European Directive 98/79/EC and meets the requirements of ISO 13485.

It is intended for trained personnel in medical laboratories authorized in good laboratory diagnostic practice and or in accordance with the recommendations of the Regional Health Agency with qualified personnel trained in molecular biology techniques, in particular real-time PCR. Laboratories are required to report all results to the competent health authorities.

SARS-CoV-2 RT-qPCR DETECTION KIT (Appolon Bioteck, Reference KSC2-AB69) is an in vitro diagnostic kit for highly specific, sensitive and rapid qualitative detection of SARS-CoV-2 nucleic acids (RNA) present in biological samples in approximately 1 hour.

The RT-qPCR DETECTION KIT has been optimised and validated to detect SARS-CoV-2 RNA in nasopharyngeal and salivary samples. These samples can be acquired from a variety of sources including symptomatic patients with SARS-CoV-2 infection, asymptomatic infected patients, patient screening campaigns implemented by the health authorities, and also from those who have had contact with an infected individual.

Positive test results are indicative of the presence of SARS-CoV-2 pathogen RNA and will amplify two separate detection target signals corresponding to two genes from the SARS-CoV-2 genome, and a third signal originating from a sample control gene used to demonstrate successful sample handling and sample processing.

The medical management of the patient should be combined with clinical observations and biological data, patient history, and epidemiological information available elsewhere. Positive results can in no way rule out bacterial infection or co-infection with viruses other than SARS-CoV-2. Negative results do not exclude infection with SARS-CoV-2. They should not be used as the sole basis for decision-making in the management of patients.

III. Test principle

SARS-CoV-2 DETECTION KIT is based on the amplification and detection of target RNA from two specific genes (ORF 1ab and N gene) present in the SARS-CoV-2 genome by reverse transcriptase polymerase chain reaction (RT-qPCR). The detection assay relies first on the conversion of target RNA into cDNA by reverse transcriptase enzymes, followed by precise amplification of target cDNA by Taq polymerase to generate sensitive and specific fluorescence detection. A 5′ exonuclease activity of Taq polymerase enzyme will degrade fluorescently labelled gene specific hydrolysis probes included in the reaction mix once hybridised to their specific detection target genes. This degradation emits a fluorescence proportional to the quantity of the specific gene detection target that can be optically monitored in real-time during the assay using a real-time PCR thermal cycler. The kit consists of a single and easy to use master mix solution of RT-qPCR mix containing hydrolysis TaqMan™ probes specific for the ORF 1ab gene and the N gene of SARS-CoV-2 pathogen labelled with the fluorochromes FAM and HEX, respectively, and also a human RNase P endogenous control gene labelled with fluorochrome Cy5. Also supplied in the kit are two test assay efficacy control tubes, a positive control solution (inactivated SARS-CoV-2 virus) used to verify the detection signal of pathogen RNA, and a negative extraction control solution (qualified human cell line without the presence of SARS-CoV-2 or other viral agents) to verify detection of the endogenous control gene.

IV. Main components

Table 1 Composition of SARS CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69)

Product name	Composition	Quantity	Volume / tube	Target (Fluorescence)	Storage	Conservation
SARS-CoV-2 RT-qPCR Mix	• OF1ab and N SARS-COV-2 specific primers and probes for • RNase P (human) specific primers and probe . • Tris-HCl, KCl, (NH4) 2SO4, MgCl2, dNTPs, UDG… • Reverse transcriptase and Hot start *Taq* DNA polymerase enzymes.	1 tube (96 tests) **Translucent cap**	1500 µL	**ORF1ab (FAM)** **N (HEX)** **RNase P (Cy5)**	-25 / -15 °C	12 months
Negative control	RNA from a qualified human cell line free from SARS-CoV-2 or other pathogens, in a viral inactivation and preservation solution	1 tube (96 tests) **Blue cap**	400 µL			
Positive control	Quantified Sars-CoV-2 RNA and qualified human cell line RNA free from SARS-CoV-2 or other pathogens, in a viral inactivation and preservation solution	1 tube (96 tests) **Red Cap**	400 µL			

The positive and negative controls allow for evaluation of sample preparation and to demonstrate successful extraction and amplification of sample target nucleic acid and also the absence of inhibitors in each RT-qPCR reaction.

V. Storage conditions and shelf life

The kit should be stored at -20°C ± 5°C, and is stable for the period stated on the kit label.
Repeated freezing and thawing should be avoided as this can impact on assay sensitivity and should not be repeated ≥3 times.

VI. Additional materials (not included)

VI.1. Viral collection and transport equipment

- Nasopharyngeal sample collections:
    - Sterile dry swabs
    - Viral transport and inactivation medium
    - Saliva collection kits containing a collection tube or sampling pipette
    - tube containing ready-to-use inactivating buffer

MTL Viral Transport Medium (Appolon Bioteck; MTL02-AB69) is recommended for use with SARS-CoV-2 RT-qPCR DETECTION KIT. For any other transport medium, please follow the supplier's recommendations.

VI.2. RNA extraction:

SARS-CoV-2 Magnetic Bead RNA Extraction Systems can be used with SARS-CoV-2 RT-qPCR DETECTION KIT according to supplier recommendations. For use with other types of RNA extraction kits please follow manufacturers recommendations and ensure RNA yields are suitable for qPCR.

VI.3. Real time PCR:

Line-Gene 9600 Plus Real-time PCR Detection System, QuantGene 9600 Real-time PCR Detection System, AGS 4800 Real-time PCR Detection System, CFX96 Touch™, LightCycler 480 System SP

VI.4. Laboratory equipment:

- Freezer at -20°C, Refrigerator + 4°C
- Disposable powder-free gloves
- Lab coat
- Centrifuge for 1.5ml and 2.0ml plate or tubes
- Vortex
- Adjustable pipettes P10, P20, P200, P1000
- Sterile filter tips for 10 µl, 20 µl, 200 µl, 1000 µl
- 1.5 ml Eppendorf microtubes or equivalent
- Microtubes, strips or PCR plates 96 positions 0.2 ml
- Optical films specific for PCR (or caps if they are strips)
- Microbiological Safety Station for handling class I or II
- Cold Rack or crushed ice

VI.5. Reagents

- MTL viral RNA transport and extraction kit (Appolon Bioteck, MTL02-AB69)
- NX-48S Viral NA Kit for Covid-19 (Appolon Bioteck, VN143) or equivalent
- H_2O for molecular biology use
- DNA-RNA decontamination products

VI.6. Specimen requirements

- Nasopharyngeal samples
- Saliva samples

VI.7. Sampling methods

It is highly recommended to follow the recommendations of the competent health authorities and treat all samples as being potentially infectious. For optimal sampling methods follow supplier instructions of the selected sampling kit. Instructions are given bellow for sample transportation:

- **Nasopharyngeal swab sample**

For optimum test sensitivity samples should be processed routinely. A reliable laboratory refrigerator for short term storage (7 days) can also be used. Samples to be dispatched by mail should be done so immediately to avoid sample degradation and reduced test sensitivity.

1- Immediately Immerse sampled swab in MTL viral transport medium (MTL02-AB69).
2- Rotate the swab to release the biological material in the MTL collection tube
3- Remove the swab from the tube and dispose following local guidelines
4- Carefully close the tube with the dedicated cap.

- **Saliva sample**

1. Collect the salivary sputum using the selected saliva collection kit
2. Transfer between 50 µl and 150 µl of saliva sample in MTL viral transport medium (MTL02-AB69)
3. Carefully close the tube with the dedicated cap.

VII. Precautions for use

VII.1. General precautions

- Always wear gloves when handling reagents of the SARS-CoV-2 RT-qPCR DETECTION Kit. In case of contact with skin, wash immediately with water.
- Prepare samples using a microbiological safety cabinet, and follow local safety procedures for waste disposal of contaminated product or infectious sample.
- Unused SARS-CoV-2 RT-qPCR DETECTION Kit reagents can be considered non-hazardous and disposed of according to usual laboratory procedures.
- Do not use the SARS-CoV-2 RT-qPCR DETECTION Kit after their expiration date.

VII.2. Special precautions for molecular biology

- It is advisable that amplification procedures require controlled arrangements and qualified trained personnel to avoid cross-contamination and complications associated with correct interpretation of sample results.
- The three steps of the procedure below should be carried out in separate rooms to reduce the risk of contamination.
- The passage from one room to another should flow in one direction starting in the sampling area and moving towards the area for reagent and PCR plate preparation and finishing in the area for PCR amplification.
- Never introduce an amplified product into the reagent or sample preparation rooms.
- Finished reaction tubes and pcr plates should be disposed of immediately after a PCR assay has finished, and this waste flow should be kept separate from all other laboratories.
- **There should be no circumstance whereby RT-qPCR reaction tubes or plates should be opened after a PCR assay is finished. This is essential to avoid laboratory contamination.
- Lab coats, pipettes and other small laboratory equipment must be dedicated for each work area. Never exchange equipment from one area to another, as this could contaminate the laboratory.
  Always use sterile aerosol-resistant pipette tips.
- Maintain an RNase-free environment with good housekeeping and decontamination practices.
- RT-qPCR tests should be protected from direct light during set-up to avoid probe degradation and erroneous amplification results.

Important:

- It is not advisable to vortex the RT-qPCR mastermix. Mix by hand or by using a pipette.
- Do not substitute reagents between different batches.
- Do not substitute reagents with those of other manufacturers.
- Do not use kit reagents if received thawed.
- Reagents must be completely thawed using a cold block (+ 4 ° C) before use.
- Defrosting is complete in 15 min. Keep reagents on the cold pack throughout handling.
- Wear disposable gloves

VIII. Preparing samples, transporting and storing

Important:

- Improper sampling, sample handling, transportation and storage conditions can lead to erroneous results.
- Samples must be taken according to the laboratory instructions
- The transport of samples must be carried out in compliance with local regulations.
- If samples are transported in a transport medium other than MTL (MTL02-AB69), we recommend that you follow the supplier's recommendations following the guidelines for molecular biology applications.
- The performance of the SARS-CoV-2 RT-qPCR DETECTION KIT has been validated for use with nasopharyngeal swabs and saliva swabs, and salivary sputum liquid sample collected in MTL transport medium (MTL02-AB69).
- The volume of the saliva sample collected must be between 50 µl and 150 µl.

MTL medium allows the collection, inactivation, viral membrane lysis and preservation of viral nucleic acids during transport at + 4 ° C. The recommended storage conditions for nasopharyngeal and salivary samples in MTL medium (MTL02-AB69) are:

- 7 days at + 4 ° C
- 6 months at -20 ° C (with a maximum of 3 freeze / thaw cycles).

IX. SARS-CoV-2 RNA Extraction Protocol

IX.1. RNA Extraction by chemical lysis

Before collection, record the patient's identifiers on the MTL transport tube (Appolon Bioteck, MTL02-AB69)

Nasopharyngeal swabs collected on swabs:

1. Place the swab in the sample collection tube containing 1 ml of MTL transport medium. Take care not to contaminate the outer surfaces of the sample tube.
2. Rotate the swab in solution to disperse and mix the biological sample material into MTL.
3. Remove the swab from the tube and dispose in a specific bin for hazardous biological materials

5. Carefully close the tube with the dedicated cap.
6. Vortex the sample for 1 minute.
7. Leave sample for 5 minutes and repeat a further 3 times.

Saliva samples:

1. Place 50µL to 100µl of saliva in 1 ml of MTL medium without touching the edges of this medium.
2. Carefully close the tube with the dedicated cap.
3. Vortex the sample for 1 minute.
4. Leave sample for 5 minutes and repeat a further two times.

IX.2. RNA extraction using magnetic beads

Extraction of SARS-CoV-2 RNAs can be performed by an automated magnetic bead system or equivalent according to manufacturers protocol.

X. Real time RT-PCR protocol

Important:

The RT-qPCR assay has been validated for testing samples individually. Each sample will require 5µl of extracted test sample RNA and 15µl of RT-qPCR reaction master mix. It is important to add RT-qPCR reaction mix first into sample PCR tubes/wells to reduce aerosol contamination during assay setup.

1. Establish the total number of required reactions including all samples and controls and produce an assay plan specifying tube locations and identities
2. Thaw the reagents and for each RT-qPCR series, predict the number of reactions as follows:
3. • 1 reaction for each sample (patient) of extracted RNA to be tested
4. • 1 reaction for the positive control
5. • 1 reaction for the negative extraction control.
6. • 1 negative control reaction of PCR without RNA (molecular biology grade water)
7. In a dedicated RT-qPCR workstation pipette 15 µl of RT-qPCR reaction mixture into each PCR sample tubes
8. Pipette 5 µl of sample RNA into corresponding sample tube/well according to the plate plan.
9. Close or cover all PCR sample tubes before addition of assay controls (if possible)
10. Add 5 µl of the positive control material to the positive control well and close/cover.
11. Place 5 µl of the negative extraction control in the negative control well and close/cover.
12. Add 5 µl of the negative PCR control without RNA.
13. Ensure all reaction wells are closed
    a. Recommended, centrifuge PCR plate for 30s to eliminate sample bubbles and to thoroughly combine sample and reaction mix in each well for optimum amplification conditions
14. Transfer immediately to a real-time PCR machine for testing. Failure to do so could result in degradation of RNA and complications with result interpretation.

    Note: For each RT-PCR assay, it is essential to include the assay kit controls to qualify each sample assay test result, and it is also advisable to include a non-template control consisting of molecular biology quality water, qualified free of DNA, RNA and RNase

RT-qPCR amplification profile:

The thermal amplification profile is shown in Table 3. Deviation from this protocol can lead to unreliable and unvalidated amplification reactions results;

4. Program the thermal cycler as shown in Table 3

Table 2 Volumes of reaction mix and sample to dispense per well of RT-qPCR

Product	Sample volume / well for each sample	Volume / well Negative extraction control	Volume / well Positive extraction control	Volume / well PCR negative control
SARS-CoV-2 RT-qPCR Mix	15 µl	15 µl	15 µl	15 µl
Extracted RNA or lysate from test sample	5 µl	-	-	-
RNA positive extraction control	-	-	5 µl	-
RNA negative extraction control	-	5µl	-	-
Molecular biology grade water	-	-	-	5 µl
Total reaction volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**

Table 3 RT-qPCR program

Steps	time	Temperature	Cycles	Fluorescence acquisition
Reverse Transcription (RT)	10 minutes	50°C	1	Not applicable
Taq polymerase activation	10 minutes	95°C	1	Not applicable
Amplification	5 seconds	95°C	45	FAM HEX Cy5
	5 seconds	59°C		

XI. Results analysis

XI.1. Fluorescence Reading channels

SARS-CoV-2 RT-qPCR DETECTION Kit allows the detection of the ORF 1ab and N genes of SARS-CoV-2 and the human RNAse P gene (endogenous control). Figure 1 shows example amplification profiles of each of these target genes when viewed individually.

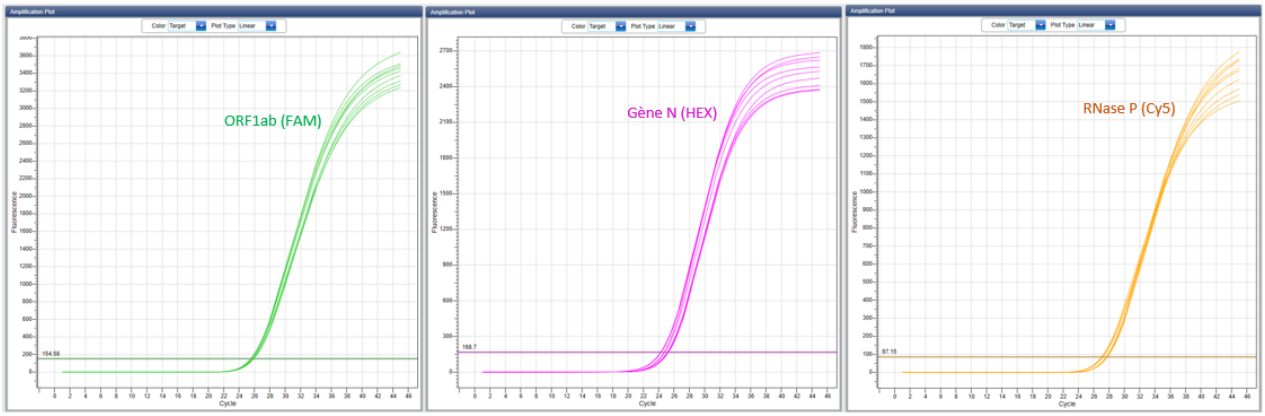

Figure 1 Amplification profiles of target genes tested by SARS-CoV-2 RT-qPCR DETECTION KIT(KSC2-AB69). The ORF 1ab gene probe emission uses 494 nm "FAM". The N gene probe emission uses 530 nm "HEX" and the RNase P gene probe emission uses 650 nm "CY5".

XI.2. RT-qPCR validation of positive and negative controls

For correct interpretation of sample test results using the SARS-CoV-2 DETECTION KIT, it is essential that the amplification results from all control reactions are closely examined and meet the criteria listed in Table 4.

Table 4 Criteria for validating an RT-qPCR series

	FAM Canal (ORF1ab)	Canal HEX (Gène N)	Canal CY5 (RNase P)	Statut de l'analyse
Positive Control	22 < Ct < 32	22 < Ct < 32	22 < Ct ≤ 35	Validated PCR
Negative Control	Ct > 40	Ct > 40	22 < Ct ≤ 35	Validated extraction

XI.3. Results interpretation

Each sample must be analyzed individually. The absence of inhibition and demonstration of successful extraction of sample nucleic acid is verified by the presence of an amplification curve in the 650 nm channel (Cy5) corresponding to the endogenous control (RNase P gene). Signal intensity in each channel is uniquely proportional to the quantity of amplified RNA present in each biologically variable sample. Representative sample amplification result are summarized in Figure 2.

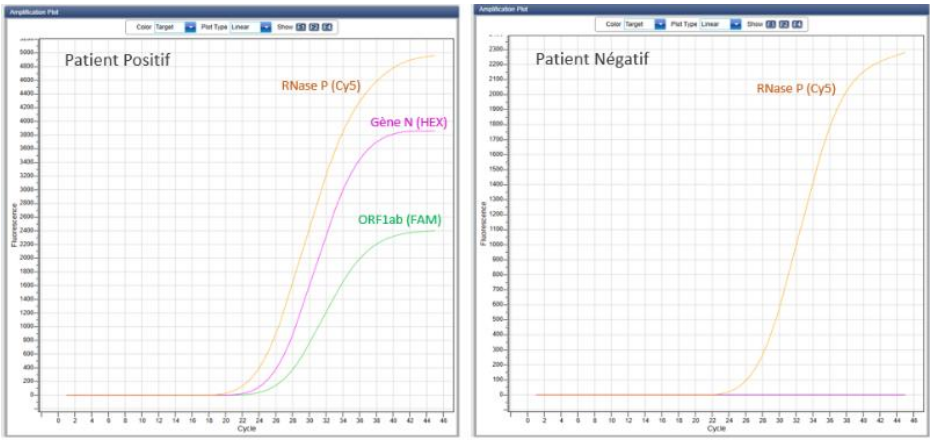

Figure 2 Example of valid RT-qPCR results for the detection of SARS-CoV-2 sample RNA. A positive detection (left) with sigmoidal amplification curves corresponding to the ORF1ab, N and RNaseP genes in the FAM, HEX and Cy5 channels,

respectively. A negative detection result (right) showing a single amplification curve corresponding to the RNaseP gene in the Cy5 channel.

The Ct cut-off values for the SARS-CoV-2 DETECTION KIT are set at 40 for the ORF1ab gene and 38.5 for the N gene. A CT value of 35 has been established for the RNaseP gene, and therefore values above 35 are considered suboptimal either from poor sample acquisition or PCR inhibition, and should be interpreted with caution. The biologist should examine the amplification curves of each SARS-CoV-2 target tested before reporting the results. The Ct values allowing the interpretation of the results are shown in Table 5.

Table 5 Interpretation of RT-qPCR results for the analyzed sample.

ORF 1ab gene *FAM Channel*	**N gene** *HEX Channel*	**Endogenous RNase P control** *CY5 Channel*	**Result interpretation**
Ct ≤ 40	Ct ≤ 38.5	Ct ≤ 35	Positive result
Ct > 40	Ct > 38.5	Ct ≤ 35	Negative result
Ct ≤ 40	Ct ≥ 38.5	Ct ≤ 35	Questionable result
Ct > 40	Ct ≤ 38.5	Ct ≤ 35	Questionable result
Ct > 40	Ct > 38.5	Ct > 35	Invalid result

Positive result

Positive samples for SARS-CoV-2 will produce sigmoidal amplification curves in the FAM channel with a Ct ≤ 40 and for HEX with a Ct ≤ 38.5. The amplification curve in the Cy5 channel shows a Ct value ≤ 35.
In some cases, fluorescence in the Cy5 channel may be absent while the two amplification curves in the FAM and HEX channels are evident. The result is considered positive.

Negative result

Negative samples for SARS-CoV-2 should not generate sigmoidal amplification curves in the FAM and HEX channels. An amplification curve in the Cy5 channel of Ct≤35 is expected and is necessary to validate the sample acceptability criteria. Ct values in FAM Ct > 40 and HEX Ct > 38.5 will sometimes be observed with valid Ct <35 for Cy5. These samples can be called negative.

Questionable result

The results may be considered questionable when the patient presents one of the following results:

1 Only one target among those sought is detected (detection only of the ORF 1ab gene or of the N gene)

2 When the target in the FAM channel has a Ct ≤ 40 and the target in the HEX channel has a Ct ≥ 38.5

3 When the target in the FAM channel has a Ct > 40 and the target in the HEX channel has a Ct ≤ 38.5

When the results are questionable, we recommend that you repeat the test and if the results remain the same, the patient should be considered positive for SARS-CoV-2.

Invalid result

The results are considered invalid in the following situations:

1 No amplification curve for the ORF1ab, N and RNase P genes (No fluorescence of FAM, HEX and Cy5)

2 No amplification curve for the ORF1ab and N genes (Absence of FAM and HEX fluorescence) with a Ct> 35 for the RNase P gene (Cy5).

3 When the results are invalid, we recommend that a dilution of sample is performed prior to retesting in case of sample inhibitors.

XII. Limits

- Use of SARS-CoV-2 DETECTION KIT is restricted to personnel trained in molecular biology procedures and techniques.
- Failure to follow the instructions in this User Manual may lead to erroneous results.
- The performances of the SARS-CoV-2 DETECTION KIT have been established on salivary and nasopharyngeal samples in an MTL transport and lysis medium (MTL02-AB69). Samples should be collected, transported and stored under appropriate procedures and conditions. Failing this, the ability of the Kit to detect target sequences of interest may be affected.
- Extraction and amplification of nucleic acids should be performed using the materials and methods listed in this user manual. For any other material not mentioned in this manual, we recommend evaluating its effectiveness by an internal laboratory validation procedure.

False negative results may come from:

- Non-compliance with the procedures for collecting samples
- Non-compliance with the conditions of transport or storage of samples
- Presence of PCR inhibitors
- Use of unauthorized or expired reagents
- Use of reagents not stored under the conditions specified in the user manual
- Presence of new mutations in the genome of the SARS-CoV-2 virus not listed to date
- Non-compliance with the instructions for using the kit

False positive results may come from:

- Cross contamination between samples during sample handling
- Labelling errors
- Contamination with the positive control during handling

A negative result does not prevent subsequent infection with the SARS-CoV-2 virus and should not be the sole basis for patient management decisions. A negative result should be combined with clinical observations, patient history, epidemiological background.

XIII. Analytical performance of the SARS-CoV-2 DETECTION KIT

The performances described below have been obtained and validated with MTL chemical extraction systems (MTL02-AB69).

XIII.1. Primers and Probes Specificities

XIII.1.1. Inclusivity

The sequences of the primers and probes used in the SARS-CoV-2 DETECTION KIT are those published and recommended by the CDC of China [1]. Sequence homology of more than 90% with all strains of the new Coronavirus involved in severe acute respiratory syndrome (sense primers, reverse primers or probes) including the new SARS-CoV-2 variants (20I / 501Y.V1, 20H / 501Y.V2 and 20J / 501Y.V3). SARS-CoV-2 DETECTION KIT is capable of detecting all strains and variants known to date of SARS-CoV-2.

XIII.1.2. Exclusivity and cross-reaction in silico

The sequences of the primers and probes used show no homology with other viral strains (42 organisms including Coronaviruses) or bacterial strains of the ORL sphere

XIII.1.3. Exclusivity and cross-reaction result using RT-qPCR

The exclusive and cross-reaction analysis of the primers and probes on a large panel of viral and bacterial strains showed no homology with other viral (including Coronavirus) or bacterial strains tested. No cross-reaction with pathogen sequences other than those sought by SARS-CoV-2 DETECTION KIT has been observed.

XIII.2. Analytical sensitivity

XIII.2.1. Analytical sensitivity on nasopharyngeal swabs samples

The detection limits (LoD) for each target tested for SARS-CoV-2 by SARS COV-2 DETECTION KIT on nasopharyngeal swabs are set at:

- **LoD for the ORF1ab gene: 50 genome copies / RT-qPCR**
- **LoD for the N gene: 10 genome copies / RT-qPCR**

XIII.2.2. Analytical sensitivity on saliva samples

The detection limits (LoD) for each target tested for SARS-CoV-2 by SARS COV-2 DETECTION KIT on saliva samples are set at:

- **LoD for the ORF1ab gene: 100 genome copies / RT-qPCR**
- **LoD for the N gene: 50 genome copies / RT-qPCR**

XIII.3. Interfering substances effects

No influence of interfering substances false result or inhibiting effect of PCR reaction was observed on the effectiveness of the SARS-CoV-2 DETECTION KIT (detection of false negatives, false positives or presence of inhibition).

XIII.4. Clinical performance of the SARS-CoV-2 DETECTION KIT (Reference KSC2-AB69)

XIII.4.1. Clinical performance on nasopharyngeal samples

The clinical evaluation of the SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69) was carried out with nasopharyngeal samples from patients tested in two French laboratories. The results obtained by SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69) were compared with those of the two laboratories as well as with the results obtained with a Kit marked CE IVD (Daan Gene / Appolon Bioteck, Reference DA0930) and referenced by the ANSM.

The study included 104 nasopharyngeal swabs positive and 102 negatives for SARS-CoV-2, respectively. The obtained clinical samples were tested blind to generate the positive (PPV) and negative (VPN) predictive values, sensitivity and specificity of the SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69). The summary results of the clinical study of SARS-CoV-2 DETECTION KIT on nasopharyngeal swabs (Appolon Bioteck Reference KSC2-AB69) are listed in table (6).

Table 6 Summary of the clinical performance results of the SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69) on nasopharyngeal swabs

Appolon Bioteck KSC2-AB69 / NASOPHARYNGEAL SWABS									
Sensibility	**95% IC**	**Specificity**	**95% IC**	**Précision**	**95% IC**	**VPP**	**95% IC**	**VPN**	**95% IC**
100%	96,52% - 100%	100%	96,45% - 100%	100%	98,23% - 100%	100%	-	100%	-

XIII.4.2. Clinical performance on saliva samples

The clinical evaluation of the SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69) was carried out with saliva samples from patients from a French laboratory. The results obtained by SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69) on these saliva samples were compared with those obtained by this laboratory on the nasopharyngeal samples taken from those same patients as well as with the results obtained with a CE IVD marked kit (Daan Gene, Reference DA0930).
The study included 53 nasopharyngeal swabs that tested positive and 170 nasopharyngeal swabs tested negative for SARS-CoV-2 with the CE IVD marked kit (Daan Gene, DA0930) and the reference laboratory.

The results obtained on these reference nasopharyngeal samples were compared with the results obtained on saliva samples from the same patients with the SARS-CoV-2 DETECTION KIT (Appolon Bioteck, KSC2-AB69).
The obtained clinical saliva samples were tested blind to generate positive (PPV) and negative (VPN) predictive values, sensitivity and specificity of SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69).

The summary results of the SARS-CoV-2 DETECTION KIT clinical study on saliva samples (Appolon Bioteck Reference KSC2-AB69) are listed in Table 7.

Table 7 Summary of the clinical performance results of the SARS-CoV-2 DETECTION KIT (Appolon Bioteck Reference KSC2-AB69) on salivary samples

Appolon Bioteck KSC2-AB69 / Saliva samples									
Sensibility	**95% IC**	**Specificity**	**95% IC**	**Précision**	**95% IC**	**VPP**	**95% IC**	**VPN**	**95% IC**
100%	93,28% - 100%	99%	96,78% - 99,99%	100%	97,54% - 99,99%	99%	88,25% - 99,73%	100%	-

XIV. Troubleshooting resolution

XIV.1. Amplification troubleshooting

Possible causes	Solutions
Stability of the amplification mix	• Avoid multiple freezing / thawing cycles • Check that the amplification master mix has been refrozen at -20°C immediately after use • Use a cold block (+ 4°C) when distributing the PCR master mix and samples. • RT and Taq polymerase enzymes are sensitive to temperature variations. Do not leave at room temperature. • Check the expiration dates of the lot used.
Non-compliance with the conditions for collecting, transporting and storing the sample	• Follow the instructions for preparing, transporting and storing samples. • Check the time between sample collection and analysis by RT-PCR.
Problem during nucleic acid extraction	• Check that the samples are homogenized before sampling for the extraction step • Verify the protocol and the equipment used with sample extractions • Always carry out preventive maintenance on automatic extraction systems according to the manufacturer's recommendations.
Reagent or sample dispensing error	• Make a plate plan to secure transfers • Check pipette calibration • Ensure that the amplification solution, controls and samples are homogenized before their distribution into the PCR plate wells
Thermal cycler programming error	• Ensure all the thermal cycler programming parameters (detection channel and mode, number of cycles, temperature, time, reaction volume, etc.) are correct.
Amplification problem	• Perform calibration maintenance on PCR devices as recommended by manufacturer • Ensure that the PCR plate is well sealed with the optical film • Use recommended plastic consumables
Error of result analysis	• Check amplification threshold lines for each target and adjust as necessary according to standard practice for molecular biology • Analyze sample amplification curves separately for each patient • Check the fluorescence intensity of amplification curves
Result misinterpretation	• Ensure that the run validation criteria are met as stated in the manual • Ensure that the thermocycler is validated for use with KSC2-AB69 RT-qPCR

XIV.2. Contamination problem

Cause	Solutions
Contamination during the experiment	• Decontaminate all small laboratory equipment using DNase RNase solutions. *Proceed with caution • Change equipment and consumables (water, PCR reagent, thermocycler, laboratory etc.)
Reagent or sample distribution error	• Create a sample plate plan • Check the pipette calibration • Ensure that the amplification mixes, controls and samples are homogenized before use

XIV.3. Inhibited samples

Cause	Solutions
Problem during extraction	• Check that the samples are well homogenized before sampling for nucleic acid extraction. • Check sample elution appears consistent • Repeat sample extraction process. • Perform preventive maintenance on automatic extraction machines in accordance with the manufacturer's recommendations.

XV. Bibliography

[1] Na Zhu, Dingyu Zhang, M.D., Wenling Wang, Xingwang Li, M.D., et al. 2020: A Novel Coronavirus from Patients with Pneumonia in China, 2019 ; N ENGL J MED 382;8.

[2] Kim, J.M., Chung, Y.S., Jo, H.J., Lee, N.J., Kim, M.S., Woo, S.H., Park, S., Kim, J.W., Kim, H.M., and Han, M.G. (2020). Identification of Coronavirus Isolated from a Patient in Korea with COVID-19. Osong Public Health Res. Perspect. 11, 3–7.

[3] Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., Si H.R., Zhu Y., Li B., Huang C.L. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–273.

[4] Dongwan Kim, Joo-Yeon Lee, Jeong-Sun Yang, Jun Won Kim, V. Narry Kim, and Hyeshik Chang The Architecture of SARS-CoV-2 Transcriptome. Cell. 2020 May 14; 181(4): 914–921.e10.

XVI. Symbols and logos used

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use
	Caution		Keep dry
	Do not use if package is damaged		Keep away from sunlight
	Date of manufacture	LOT	Batch code
	Manufacturer		Biological risks
CE	the product meets European requirements		

APPOLON BIOTECK

205 rue des frères Lumière
69 970 CHAPONNAY
FRANCE
+33 (0)4 37 57 00 54
contact@appolonbioteck.com
www.appolonbioteck.fr