# AZURESEQ 4 CE QPCR KIT FOR 200 REACTIONS

## For one step direct detection of SARS COV-2, Influenza A and Influenza B

# INSTRUCTION FOR USE

In Vitro Diagnostic Medical Device

REF **AzureSeq 4-200 CE**

DOCUMENT VERSION V02

06/10/2022

CE

IVD

# Contents

# 1. Warnings and precautions

## 1.1. Symbols used in the Instructions for Use

Caution; General

Warning; Biological risk

Prohibition

Warning; Low temperature / Freezing conditions

## 1.2. Warnings and Precautions Regarding Performance

Good laboratory practice (GLP) is essential for the proper execution of the test. Due to the sensitivity of the device, care should be taken when handling samples and materials to ensure that reagents and amplification mixtures are not contaminated.

Read the Instructions for Use. Deviation from the described activities can affect the optimal performance! Misconduct can lead to mistakes.

In the event of errors arising from non-compliance with the protocol steps described in the Instructions for Use, the manufacturer shall not be liable for the validity of the results.

Tests shall be carried out with properly maintained and calibrated qPCR instruments

A maintained instrument will be calibrated for dyes recommended by the supplier of the instrument.

Repeated freezing/thawing of the contents of the product should be avoided as this may lead to degradation of the reagents and thus a decrease in sensitivity.

Dissolve and dilute the reagents only as described in the Instructions for Use.

Only use DNase and RNase free consumables.

False negative results may occur if samples contain substances which may inhibit PCR reaction or diluting the sample.

If the patients' medical conditions allow, avoid use of any treatment* before specimen collection, which may interfere with the PCR reaction or causing dilution of the sample. When such treatments cannot be avoided, pay close attention to the detection of the internal control during the interpretation of the test results to prevent reporting false negative results.

Follow the Instructions for Use during specimen collection and sample handling!

Swab specimens should be collected using only swabs with a synthetic tip and plastic shaft.

Samples can be stored at 2-8 °C for 48 hours from sampling time.

Repeated freezing/thawing of the sample should be avoided. If a sample is retained for retesting, it is recommended that they be aliquoted before freezing.

*e.g., nasal sprays or gels, systemic or local antiviral or antibacterial agents, throat lozenges, etc.

Do not use the product past the expiration date given on the label!

Do not use the product if you experience any damage (e.g. broken components, loose caps)!

Do not use sampling devices with calcium alginate or cotton swabs with wooden shafts, as these may contain substances that inactivate the virus and inhibit PCR testing.

Do not use quantity of reagents other than those specified in the Instructions for Use.

Don't replace or mix AzureSeq reagents with other manufacturer's products!

Do not mix and match vials between kits. Vials from Primers and Probes Kits bearing different catalog numbers or are from different productions (different LOT numbers) can NOT be used interchangeably.

## 1.3. Warnings and Precautions Regarding Environmental Conditions

Components to be stored at -20°C.


Transport of the product is carried out on dry ice. Components arrive frozen.

For storage longer than 2 days, samples must be kept at -70 °C!

## 1.4. Warnings and Precautions Regarding the Chemical or Biological Risks of the Device

When carrying out the test, take general precautions!

Wear protective equipment when carrying out processes!

Treat samples, materials and instrumentation as potentially infectious!

Use disinfectant to clean and disinfect the areas used during the processing of samples!

Wash your hands thoroughly after removing gloves!

Discard used gloves into the hazardous waste bin!

Disinfect and treat used samples, reagents, and other potentially contaminated materials in accordance with local regulations

Do not smoke, drink, eat or use cosmetics in areas where samples or product components are handled!

The SDS sheets can be downloaded from the following address:
https://www.omixon.com/products/azureseq-200-ce/msds/

## 1.5. Warnings About Special Conditions Related to Use of the Device

All instruments must be operated and maintained in accordance with the manufacturer's instructions.

Each workplace must be equipped with its own pipettes and the necessary auxiliary materials and equipment

## 1.6. Warnings About Specific Qualification/Training Conditions for Using the Device

The AzureSeq 4 CE qPCR Kit for 200 Reactions is designed for use by qualified clinical laboratory staff, who have been instructed and trained specifically for the use of real-time PCR and *in vitro* diagnostic procedures.

## 1.7. Notice For the User to Report Serious Incidents Involving the Device

The user shall report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established

| | |
|---|---|
| **GTIN Code of the device:** | **5999565781231** |
|  Omixon Biocomputing Ltd | Kaposvár u. 14-18.<br>Budapest<br>H-1117<br>Hungary |
| **Contact details of competent authorities:** | https://ec.europa.eu/health/medical-devices-sector/new-regulations/contacts_en |

# 2. Intended use

The AzureSeq 4 CE qPCR Kit for 200 Reactions qPCR test is intended for the qualitative detection of nucleic acid from 2019-nCoV, Influenza A, and Influenza B, in nasopharyngeal (NP) and oropharyngeal (OP) swabs from individuals with signs and symptoms of infection who are suspected of COVID-19 or influenza.
The AzureSeq 4 CE qPCR Kit for 200 Reactions is intended for use by qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
Positive results are indicative of active infection.
Negative results do not preclude 2019-nCoV or Influenza infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

# 3. Principle of the Method

Transport media exposed to a nasopharyngeal or oropharyngeal swab is heat-inactivated at 95°C. A sample of the transport media is transferred, and viral RNA is reverse transcribed into cDNA with the AzureSeq 4 CE qPCR Kit for 200 Reactions master mix. Sample input and elution volumes are system dependent. Probes present in the master mix anneal to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at specific PCR cycles. AzureSeq 4 CE qPCR Kit for 200 ReactionsSARS-CoV-2, Influenza A, and Influenza B viruses and functional RNaseP gene reactions generate fluorescent signals of different wavelengths, which are detected by multiplex qPCR systems through the right channels.

Target sequences of viruses to be detected have been determined by the CDC as follows:

- SARS-CoV-2: virus nucleocapside gene (N)
- Influenza A: virus Matrix gene (M1)
- Influenza B: Non-structural protein gene 2 (NS2)
- Internal operational control: RNaseP gene.

# 4. Kit Content

| Product Code | Name | Identification By Color Marking | Number Of Vials | Shipped Volume [µL] |
|---|---|---|---|---|
| OA-ITMP- MM-100 | 2X InhibiTaq Multiplex HotStart MasterMix | Orange cap | 2 | 1000 |
| OA-RTM-200 | RT Mix | Blue cap | 1 | 200 |
| OA-CPPM4-100uL | CoVi PLUS Primer/Probe Mix 4 | Green cap and brown tube | 2 | 100 |
| OA-NFW-350uL | Nuclease Free Water | Yellow cap | 2 | 350 |
| OA-CVNC-150 | CoVi Negative Control* | Colorless cap | 1 | 150 |
| OA-CVPC-150 | CoVi Positive Control | Lavender cap | 1 | 150 |
| OA-FABPC-150 | Flu A/B Positive Control | Red cap | 1 | 150 |

*Used as a negative control for all targeted virus species since it does not contain biologic components.

# 5. Shelf Life, Shipment and Storage

The expiration date provided by the manufacturer is 12 months.
The AzureSeq 4 CE qPCR Kit for 200 Reactions product is transported on dry ice. Components arrive in a frozen state. Please contact our customer service (azureseq.support@omixon.com), if any of the components do not arrive in accordance with the descriptions!
If you work in an area prone to power outages, it is recommended to use a backup generator and a temperature log to ensure that the components are properly cooled.

To avoid degradation of the reagents, the components should be stored at -20°C upon arrival.

Do not use the product past the expiration date given on the label!

Do not use the product if you experience any damage (e.g. broken components, loose caps)!

Repeated freezing/thawing of the contents of the product should be avoided as this may lead to degradation of the reagents and thus a decrease in sensitivity.

Handle used reagents and waste in accordance with local regulations.

# 6. Specimen Collection and Sample Handling

Improper sampling, transport and storage can increase the likelihood of false negative results.

## 6.1. Sampling and Collection

Based on recommendations of the 'Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing' at https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html.

Good laboratory practice is essential for the proper execution of the test. Due to the sensitivity of the device, care should be taken when handling samples and materials to ensure that reagents and amplification mixtures are not contaminated.

Read the Instructions for Use. Deviation from the described activities can affect the optimal performance! Misconduct can lead to mistakes.

Follow the Instructions for Use during specimen collection!

Swab specimens should be collected using only swabs with a synthetic tip and plastic shaft.

False negative results may occur if samples contain substances which may inhibit PCR reaction or diluting the sample.

If the patients' medical conditions allow, avoid use of any treatment* before specimen collection, which may interfere with the PCR reaction or causing dilution of the sample. When such treatments cannot be avoided, pay close attention to the detection of the internal control during the interpretation of the test results to prevent reporting false negative results.

Do not use sampling devices with calcium alginate or cotton swabs with wooden shafts, as these may contain substances that inactivate the virus and inhibit PCR testing.

*e.g., nasal sprays or gels, systemic or local antiviral or antibacterial agents, throat lozenges, etc.

When carrying out the test, take general precautions!

Treat samples as potentially contagious!

Wear protective equipment when carrying out processes!

Use disinfectant to clean and disinfect the areas used during the processing of samples!

**Oropharyngeal sampling**

Use a sterile swab to wipe the posterior pharynx, avoiding the tongue. Place swabs immediately into labeled sterile tubes containing viral transport medium. Break each applicator stick off at the score line (flocked swabs) or near the tip or cut with sterile scissors to allow tightening of the cap. Ship sample immediately on cold packs.

**Nasopharyngeal sampling**

Insert a sterile swab into nostril parallel to the palate. Swab should reach a depth equal to the distance from the nostrils to the outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Place swabs immediately into labeled sterile tubes containing viral transport medium. Break each applicator stick off at the score line (flocked swabs) or near the tip or cut with sterile scissors to permit tightening of the cap. Ship sample immediately on cold packs.

## 6.2. Sample Handling

Follow the Instructions for Use during sample handling!

When carrying out the test, take general precautions!

When handling potentially infectious samples, laboratory workers must wear appropriate personal protective equipment, including disposable gloves, laboratory cloaks/clothing, and eye protection.

Treat samples as potentially infectious!

Use disinfectant to clean and disinfect the areas used during the processing of samples!

Wash your hands thoroughly after removing gloves!

Discard used gloves into the hazardous waste bin!

Disinfect and treat used samples, reagents and other potentially contaminated materials in accordance with local regulations

Do not smoke, drink, eat or use cosmetics in areas where samples or product components are handled!

Specific instructions on how to handle clinical samples for coronavirus 2019 can also be found on the CDC's website.

## 6.3. Transport

Samples must be transported in a cooled state (cooler block/dry ice), safely sealed and treated.

Clinical samples must be transferred in accordance with local regulations and SARS-CoV-2 biosecurity standards.

## 6.4. Storage

Follow the Instructions for Use during sample storage!

Samples can be stored at 2-8 °C for 48 hours from sampling time.

Repeated freezing/thawing of the sample should be avoided. If a sample is retained for retesting, it is recommended that they be aliquoted before freezing.

The direct method can be used if the sample is not frozen and stored for less than 48 hours from sampling.

Depending on the type of sample and the Virus Transport Medium used, a special storage method or pre-treatment of the sample may be required to isolate the RNA. Please follow the manufacturer's instructions!

If the sample is stored for more than 48 hours, the workflow requires the extraction of RNA with a validated RNA isolation system.

For storage longer than 2 days, samples must be kept at -70 °C!

# 7. Materials and Equipment Necessary but Not Provided

## 7.1. Instruments

- Real-time qPCR instrument that can simultaneously use the following channels

| Virus / target | Dye / Channel | | Detection wavelength [nm] |
|---|---|---|---|
| | Recommended | Alternative | |
| **SARS-CoV-2** | **FAM** | – | 510 |
| **Influenza A** | **Quasar 670** | | 670 |
| | | Cy5 | 660 |
| **Influenza B** | **HEX** | VIC | 580 |
| | | JOE | 555 |
| | | YAKIMA YELLOW | 551 |
| **RP** | **ROX** | | 604 |
| | | Texas Red | 615 |

All four channels must be used during the measurement!

Tests shall be carried out with properly maintained and calibrated qPCR instruments
A maintained instrument will be calibrated for dyes recommended by the supplier of the instrument.

- Heated block (compatible with 1.5 mL microcentrifuge tubes used in the test, suitable for incubation at 95°C)
- Micropipette capable of handling quantities 100 µL and 1000 µL
- Multichannel pipette for handling 10 µL and 100 µL quantities
- Vortex mixer
- Centrifuge

## 7.2. Reagents

- Viral/complete RNA extraction system (for frozen samples)
- Virus Transport Medium (Clinichem, Copan, Puritan, CDC VTM, mwe Medical Wire)

## 7.3. Consumables

- Optical plate compatible with the qPCR instrument used
- Optical film compatible with the qPCR instrument used
- Disposable DNase/RNase-free sterile pipette tips (10 µL, 100 µL, and 1000 µL)
- DNase/RNase-free 1.5 ml microcentrifuge tube
- Disposable rubber gloves
- Surface disinfectant

# 8. Procedure

The AzureSeq 4 CE qPCR Kit for 200 Reactions product uses RNase P RNA as an internal control, which confirms the presence of nucleic acid in the system for each sample tested, thus ensuring the functionality of the components of the product.

For a direct workflow (without RNA isolation), follow the following protocol from step 1.

When processing RNA extracted with a conventional validated isolation system, skip points 1-4 and start the workflow at point 5 of the protocol.

## 8.1. Workflow

### 8.1.1. Sample Preparation

Recommended only when processing samples from nasal and oral mucosa dissolved in Viral Transfer Medium (VTM).

Recommended Virus Transfer Medium: Clinichem, Copan, Puritan, CDC VTM, mwe Medical Wire

Samples can be stored in Viral Transfer Medium at 4 °C for 48 hours.

1. Obtain swabbed OP/NP material in VTM.
2. Transfer 100µL of swabbed OP/NP VTM material into compatible DNase/RNase free tube.
3. Heat sample for **5 minutes at 95°C**
4. Spin the tube for 30 seconds at ~1500 x rpm. Store it on ice until needed.

### 8.1.2. Master Mix Set-Up

5. Completely thaw the CoVi Primer/Probe mix 4 (brown tube/green cap) by setting on ice for ~30 minutes. Once thawed, briefly centrifuge to collect contents at the bottom of the tube.
6. Vortex the tube at max speed for a few seconds to mix, then spin down briefly to collect contents at the bottom of tube.
7. Vortex the 2x InhibiTaq Multiplex qPCR MasterMix tube thoroughly (maximum speed, minimum speed of 3200 rpm, 2-4 seconds) and visually check for precipitation in the tube.
8. Proceed to master mix setup as shown below in a clean room or designated setup area:

Assemble a reaction mixture for 20 µL final volume:

| Component | | Volume [µl] | | End value |
|---|---|---|---|---|
| Name | Identification By Color Marking | Per reaction | Per 100 reactions | |
| 2X InhibiTaq Multiplex qPCR MasterMix | Orange cap | 10,0 | 1000 | 1X |
| Direct RTScript Mix | Blue cap | 1,25 | 125 | 1X |
| 20X Primer/Probe Mix | Green cap and black tube | 1,0 | 100 | 1X |
| Nuclease-free water | Yellow cap | 2,75 | 275 | NA |

9. Suspend the master mix with a pipette set to a volume corresponding to the added 2x InhibiTaq Master Mix, or vortex the tube for a short time and spin it down for a few seconds to get its contents to the bottom of the tube.
10. Transfer **15µL** master mix to the correct plate positions in a 96-well optical plate.
11. Measure **5 µL** samples, or positive/negative controls into the correct positions.
12. Seal the plate with foil, then vortex it for a few seconds, then spin the plate for a few seconds to get the contents to the bottom of the wells.
13. Insert the plate into the qPCR instrument and start the run according to the appropriate program.

### 8.1.3. qPCR Instrument Setup

**Recommended PCR conditions:**

| Cycling step | Stage | No. of Cycles | Temperature [°C] | Time |
|---|---|---|---|---|
| RT incubation | 1 | 1 | 50 | 15 min |
| Enzyme activation | 2 | 1 | 95 | 2 min |
| Amplification* | 3 | Minimum 40 | 95 | 3 sec |
| | | | 60 | 30 sec |

*: Record fluorescence during annealing/extension phase (60°C) step on FAM, HEX, Quasar 670 (Cy5) and ROX channels (or equivalent channels).

**Detection Wavelengths / Channels**

| Vírus / target | Dye/ Channel | | Detection wavelength[nm] |
|---|---|---|---|
| | Recommended | Alternative | |
| SARS-CoV-2 | FAM | – | 510 |
| Influenza A | Quasar 670 | | 670 |
| | | Cy5 | 660 |
| Influenza B | HEX | VIC | 580 |
| | | JOE | 555 |
| | | YAKIMA YELLOW | 551 |
| RP | ROX | | 604 |
| | | Texas Red | 615 |

All four channels must be used during the measurement!

Tests shall be carried out with properly maintained and calibrated qPCR instruments
A maintained instrument will be calibrated for dyes recommended by the supplier of the instrument.

# 9. Interpretation of results

Before interpreting the results of the measurements, it is necessary to examine the test controls. In the case of invalid controls, the result of the test is inconclusive, so the result cannot be communicated.

If one or both controls are invalid or show unexpected results, the results of that run cannot be used.

The product AzureSeq 4 CE qPCR Kit for 200 Reactions uses the RNase P gene as internal operational control. In the RNase P reaction (ROX channel), the sample should show a fluorescence growth curve that exceeds the threshold within 35 cycles (35 Ct), thus confirming the presence of the human RNase P gene. Failure to detect RNase P in any sample may indicate:

- RNA loss/degradation due to inadequate nucleic acid isolation/heat treatment.
- Poor specimen quality or loss of specimen integrity.
- Improper preparation and execution of the test.
- Reagent or equipment malfunction.

In the case of the RNase P test does not show a positive result in human clinical samples, if SARS CoV-2 and/or Influenza A and/or Influenza B test is positive, the result should still be considered valid with negative RNase P signals. It is possible that some samples do not show RNase P growth

curves due to the low cell counts in the original clinical sample. The negative RNase P signal does not exclude the presence of target viral RNA in a clinical sample.

Use the following table as a general guide to interpret results.

| Result | | | | Target RNA Detection | Test Result | Report / Additional Activities |
|---|---|---|---|---|---|---|
| InfA | InfB | SC2 [a] | RP | | | |
| + | - | - | + or - | Inf A RNA | Influenza A positive sample | Report results to sender |
| - | + | - | + or - | Inf B RNA | Influenza B positive sample | Report results to sender |
| - | - | + | + or - | SC2 RNA | COVID-19 positive sample | Report results to sender |
| - | + | + | + or - | Inf B and SC2 | Influenza B and COVID-19 positive sample | Report results to sender |
| + | + | - | + or - | Inf A and Inf B | Influenza A and Influenza B positive sample | Report results to sender |
| + | - | + | + or - | Inf A and SC2 | Influenza A and COVID-19 positive sample | Report results to sender |
| + | + | + | + or - | Inf A, Inf B and SC2 | influenza A, influenza B and COVID-19 positive sample | Report results to sender |
| - | - | - | + (Ct<35) | No target RNA | Negative sample | Report results to sender, Testing for other respiratory viruses |
| - | - | - | - (Ct<35) | Invalid test | Invalid results | Repeat test, report confirmed invalidity to the submitter, consider collecting a new specimen |

# 10. Known Limitations of the Test

The device may only be used on nasopharyngeal (NP) and oropharyngeal (OP) swabs from individuals with signs and symptoms of infection who are suspected of COVID-19 or influenza.

The use of this device requires trained, professional laboratory staff.

The diagnostic results cannot be used on their own and should be interpreted in considering other known clinical symptoms and laboratory findings.

Validating the system before each run is the responsibility of the user, it is not included in the Performance Evaluation of the Omixon Biocomputing Ltd.

The effectiveness of the direct method (RNA isolation without heat treatment) depends to a large extent on the Viral Transport Medium (VTM) used in the sample collection process. Medium other than those recommended in point 8.1.1 must be validated by the user laboratory.

A negative result does not exclude the possibility of infection, since the results depend on proper sample collection and the presence of inhibitors. The presence of PCR inhibitors is invalid or may cause dubious results. These cases require repetition of the test and/or specimen collection.

False positive results can occur for several reasons, mostly related to contamination during the treatment and preparation of the RNA sample.

The impacts of antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been fully evaluated.

# 11. Troubleshooting

| Problem | Possible Root Cause | Recommendation |
|---|---|---|
| Fluorescent intensity is weak or does not appear in the positive control | Probe degradation | Use a new probe aliquot or repeat test with a new kit LOT |
| High inconsistency in the fluorescence signals in samples | Inaccurate pipetting | Use calibrated pipettes, make sure that an equal volume of reagents is added to each wells/tube |
| Fluorescent signal is detected in the negative control reaction | Carry-over contamination | Always change tips between samples. Take care when dispensing samples, negative controls, and positive controls |
| | Contamination of the amplification master mix | Use a new aliquot of amplification mix |
| | Contamination of the extraction/preparation area | Use disinfectant to clean and disinfect the areas |
| No fluorescent signal is detected in all samples, including positive control | Probe degradation | Use new probe aliquot |
| | Thermal cycler setting error | Verify the correct program setting of the real time PCR instrument |
| | Wrongly prepared master mix, possible omitted component | Verify each component and repeat the PCR master mix preparation |
| False negative results | Presence of RT-PCR inhibitors | Use VTM from the recommended manufacturers listed in this IFU |
| | Improper sample collection | Follow validated sample collection method |
| | Failure to follow instruction for use | Read the Instructions for Use carefully before processing samples. Any deviation from procedures written here may affect optimal performance. |
| | Using unauthorized reagents | Do not substitute or mix the AzureSeq kit reagents with reagents from other manufacturers. |
| False positive reaction | Contamination between samples, mixed sample | Take extra care when handle patient specimens, use straightforward and validated traceability processes in the laboratory. |

# 12. Quality Control

It is recommended to validate the entire procedure (applying direct method or RNA isolation) and the amplification with negative and positive controls or using calibrated reference samples.

# 13. Performance Characteristics

## 13.1. Limit Of Detection (LoD)

The LoD study determines the lowest viral concentration (replica (cp) / reaction) that can be detected in at least 95% of the replicates by the AzureSeq 4 CE qPCR Kit for 200 Reactionsdevice.

The LoD values for genomic RNA of the AzureSeq 4 CE qPCR Kit for 200 Reactions:

### 13.1.1. LoD on Applied Biosystems™ 7500 Fast Dx real time PCR Instrument

| LoD [$ID_{50}$/ml] | | |
|---|---|---|
| SARS-CoV-2 | Influenza A | Influenza B |
| $1{,}01x10^{-2}$ | $5{,}05x10^{-1}$ | $7{,}98x10^{-1}$ |

## 13.2. Precision

Precision characteristics were determined using the viral controls included in the AzureSeq 4 CE qPCR Kit for 200 Reactions. Calculations are based on Ct values recorded in 12 parallel measurements carried out on Applied Biosystems™ 7500 Fast Dx real time PCR Instrument.

**1. Repeatability**

| Repeatability [CV%*] | | | |
|---|---|---|---|
| SARS-CoV-2 | Influenza A | Influenza B | Negative samples [Percentage agreement*] |
| 1,37 | 0,47 | 0,52 | 97,22 |

*For negative samples, CV% values cannot be calculated from Ct measurements.

### 13.2.1. Reproducibility

| Reproducibility [CV%*] | | | |
|---|---|---|---|
| SARS-CoV-2 | Influenza A | Influenza B | Negative samples [Percentage difference*] |
| 1,90 | 0,97 | 0,65 | 2,78 |

*For negative samples, CV% values cannot be calculated from Ct measurements.

## 13.3. Inclusivity And Exclusivity / Target Specificity and Sensitivity / Cross-Reactivity

### 13.3.1. *In silico* Analyses

**SARS-CoV-2**

The inclusivity/exclusivity of each primer and probe oligonucleotide sequence of the AzureSeq 4 CE qPCR Kit for 200 Reactionsfor the SC2 target was tested against 31,623 in the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) database by June 6, 2021.

The results confirmed a perfect match with SARS CoV-2 and close matches with the ancestors of SARS CoV 2, meaning no genome was found that was identified with a difference of more than 2 nt. The frequency of sequence differences between each primer and rehearsals is <1%, with variable sequence localization indicating that the differences are sporadic and indicate inconsistent mutations.

The homology evaluation between sarscov2 sequences available on June 6, 2021, and the CDC Influenza SARSCoV2 Multiplex Assay primers and trials shows that the likelihood of a significant reduction in reactivity resulting from sequence deviations (up to 2 nucleotides) is small and therefore the risk of false negative outcomes is low.

**SARS-CoV-2 new variants**

*In silico* analysis as well as RT-qPCR tests were performed against the currently known variants of SARS-CoV-2 virus: original alpha strain (WT), UK/SA (B.1.1.7), Delta (B.1.627.2), and Omicron variant (B.1.1.529). As predicted by the *in silico* analysis, AzureSeq reagents successfully amplify N1 and N2 targets against all tested RNA variants. N1 performance against the Omicron template, which contains a single mismatch in the N1 probe, was not affected and performed equally to the other variants.

In summary the AzureSeq reagents perform equally against all major strains of SARS-CoV-2 RNA seen in global circulation to the date of the issuance of this IFU (WT, B.1.1.7 UK/SA, B.1.627.2 Delta, B.1.1.529 Omicron). Therefore, it is expected that AzureSeq reagents will continue to amplify and detect SARS-CoV-2 Omicron variant RNA from individuals who have detectable levels of SARS-CoV-2 present in appropriately collected sample types.

**Suitability for European Seasonal Influenza Strains**

An analysis was performed to check the suitability of the primers and probes used in AzureSeq 4 CE qPCR Kit for 200 Reactions for detecting influenza A and B subtypes commonly found in Europe and to assess the phylogenetic relationship of the assay targets between the previously tested subtypes.

Based on the information presented in the report, it can be concluded that the influenza A and influenza B primers and probes used in AzureSeq 4 CE qPCR Kit for 200 Reactions are suitable for detecting common influenza A and B subtypes observed in Europe in recent years.

### 13.3.2. Laboratory Cross-Reaction Investigations

**Cross Reactions with Non-Target Coronaviruses (FDA SARSCoV2 Reference Panel)**

| Vírus | Strain | SARS-CoV-2 | Inf A | Inf B |
|---|---|---|---|---|
| Zoonotic beta coronavirus | MERS-CoV | 0,00 | 0,00 | 0,00 |
| Endemic beta coronavirus | HCoV OC43 | 0,00 | 0,00 | 0,00 |
| Endemic alpha coronavirus | HCoV 229E | 0,00 | 0,00 | 0,00 |
| Endemic alpha coronavirus | HCoV NL63 | 0,00 | 0,00 | 0,00 |
| HKU1 | HCoV HKU1 | 0,00 | 0,00 | 0,00 |

**Cross-Reactions with Target and Non-Target Influenza Viruses**

| Vírus | Host | Subtype | Virus Strain | SARS-CoV-2 | Inf A | Inf B |
|---|---|---|---|---|---|---|
| Influenza A | Human | A(H1N1) pdm09 | A/Florida/81/2018 | 0,00 | 14,00 | 0,00 |
| | Human | A(H3N2) | A/Kansas/14/2017 | 0,00 | 13,65 | 0,00 |
| | Swine | A(H1N2) v | A/Ohio/35/2017 | 0,00 | 14,82 | 0,00 |
| | Swine | A(H3N2) v | A/Ohio/13/2017 | 0,00 | 20,89 | 0,00 |
| | Equine | A(H3N8) | A/equine/Ohio/01/2003 | 0,00 | 16,63 | 0,00 |
| | Canine | A(H3N2) | A/canine/Florida/43/2004 | 0,00 | 19,58 | 0,00 |
| | Feline | A(H7N2) | A/feline/New York/16-040082-1/2016 | 0,00 | 15,90 | 0,00 |
| | Avian | A(H2N2) | A/chicken/Pennsylvania/298101-4/2004 | 0,00 | 15,67 | 0,00 |
| | Avian | A(H5N2) | A/Northern pintail/Washington/40964 /2014 | 0,00 | 16,44 | 0,00 |
| | Avian | A(H5N8) | A/gyrfalcon/Washington/41088 -6/2014 | 0,00 | 14,12 | 0,00 |
| | Avian | A(H6N2) | A/chicken/California/32213-1/2000 | 0,00 | 15,03 | 0,00 |
| | Avian | A(H7N9) | A/Taiwan/1/2017 | 0,00 | 16,86 | 0,00 |
| | Avian | A(H9N2) | A/Bangladesh/0994/2011 | 0,00 | 18,13 | 0,00 |
| Influenza B | Human | B-VIC | B/Maryland/15/2016 | 0,00 | 0,00 | 13,47 |
| | Human | B-YAM | B/Phuket/3073/2013 | 0,00 | 0,00 | 13,67 |
| Influenza C | Human | – | C/Minnesota/1/2016 | 0,00 | 0,00 | 0,00 |
| | Human | – | C/Minnesota/4/2015 | 0,00 | 0,00 | 0,00 |
| | Human | – | C/Minnesota/29/2015 | 0,00 | 0,00 | 0,00 |

### 13.3.3. Microbiological Interference Tests

Samples from high-titer preparations of 35 organisms (16 viruses, 18 bacteria and 1 yeast, see below) that represent respiratory pathogens or flora, which are often present in human respiratory samples and/or close genetic neighbors of viruses to be determined with the AzureSeq 4 CE qPCR Kit for 200 Reactions were examined.

| Pathogen | Strain | Ct | | |
|---|---|---|---|---|
| | | SARS-CoV-2 | Influenza A | Influenza B |
| *Bordetella pertussis* | Tohama I | 0,00 | 0,00 | 0,00 |
| *Candida albicans (yeast)* | 3147 | 0,00 | 0,00 | 0,00 |
| *Chlamydia pneumoniae* | CM-1 | 0,00 | 0,00 | 0,00 |
| *Corynebacterium diphtheriae* | NCTC 13129 | 0,00 | 0,00 | 0,00 |
| *Escherichia coli* | K12 | 0,00 | 0,00 | 0,00 |
| *Streptococcus pyogenes* | 7790-06 | 0,00 | 0,00 | 0,00 |
| *Haemophilus influenzae* | M15709 | 0,00 | 0,00 | 0,00 |
| *Lactobacillus plantarum* | NA | 0,00 | 0,00 | 0,00 |
| *Legionella pneumophila* | Philadelphia-1 | 0,00 | 0,00 | 0,00 |
| *Moraxella catarrhalis* | M15757 | 0,00 | 0,00 | 0,00 |
| *Mycobacterium tuberculosis* | H37Ra | 0,00 | 0,00 | 0,00 |
| *Mycoplasma pneumoniae* | PI 1428 | 0,00 | 0,00 | 0,00 |
| *Neisseria elongata* | NA | 0,00 | 0,00 | 0,00 |
| *Neisseria meningitidis* | M2578 | 0,00 | 0,00 | 0,00 |
| *Pseudomonas aeruginosa* | NA | 0,00 | 0,00 | 0,00 |
| *Staphylococcus aureus* | NA | 0,00 | 0,00 | 0,00 |
| *Staphylococcus epidermidis* | NA | 0,00 | 0,00 | 0,00 |
| *Streptococcus pneumoniae* | 249-06 | 0,00 | 0,00 | 0,00 |
| *Streptococcus salivarius* | DSM 13084 | 0,00 | 0,00 | 0,00 |
| *Human Adenovirus, type 1* | Ad.71 | 0,00 | 0,00 | 0,00 |
| *Human Adenovirus, type 7a* | S-1058 | 0,00 | 0,00 | 0,00 |
| *Human parainfluenza 1* | NA | 0,00 | 0,00 | 0,00 |
| *Human parainfluenza 2* | Greer | 0,00 | 0,00 | 0,00 |
| *Human parainfluenza 3* | C-243 | 0,00 | 0,00 | 0,00 |
| *Respiratory syncytial virus* | CH93-18b | 0,00 | 0,00 | 0,00 |
| *Human Rhinovirus A* | 1A | 0,00 | 0,00 | 0,00 |
| *Enterovirus* | Echo 6 | 0,00 | 0,00 | 0,00 |
| *Herpes Simplex virus* | KOS | 0,00 | 0,00 | 0,00 |
| *Varicella-zoster virus* | AV92-3:H | 0,00 | 0,00 | 0,00 |
| *Epstein Barr virus* | B95-8 | 0,00 | 0,00 | 0,00 |
| *Measles* | Edmonston | 0,00 | 0,00 | 0,00 |
| *Mumps* | Enders | 0,00 | 0,00 | 0,00 |
| *Cytomegalovirus* | AD-169 | 0,00 | 0,00 | 0,00 |

## 13.4. Clinical Performance

Since comparator devices were used during clinical evaluation, due to the lack of a reference method, percentage agreement measures (positive percentage agreement - PPA, negative percentage agreement - NPA and general percentage agreement - OPA) were determined against the comparator devices [9].

### 13.4.1. Percentage Agreement Measures for Bio-Rad CFX384 real time PCR Instrument

| Virus | Comparator Device | Percentage Agreement |
|---|---|---|
| SARS-CoV-2 | CDC 2019nCoV RealTime RTPCR Diagnostic Panel using the ThermoFisher TaqPath™ 1Step RTqPCR Master Mix | PPA = 100,00% |
| | | NPA = 100,00% |
| | | OPA = 100,00% |
| Influenza A | CDC Human Influenza Virus RealTime RTPCR Diagnostic Panel Influenza A/B Typing Kit using the Invitrogen™ SuperScript™ III Platinum™ OneStep qRTPCR Ki | PPA = 100,00% |
| | | NPA = 100,00% |
| | | OPA = 100,00% |
| Influenza B | CDC Human Influenza Virus RealTime RTPCR Diagnostic Panel Influenza A/B Typing Kit using the Invitrogen™ SuperScript™ III Platinum™ OneStep qRTPCR Ki | PPA = 100,00% |
| | | NPA = 100,00% |
| | | OPA = 100,00% |

# 14. References


1. COMMUNICATION FROM THE COMMISSION Guidelines on COVID-19 in vitro diagnostic tests and their performance, Brussels, 15.4.2020 C(2020) 2391 final
2. DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices Working document of Commission services - Current performance of COVID-19 test methods and devices and proposed performance criteria 16 April 2020 (working document)
3. Regulation (EC) No. 1907/2006 Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
4. Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006
5. CDC Oct. 6, 2021 CDC's Influenza SARS-CoV-2 Multiplex Assay and Required Supplies at https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex.html
6. CDC July 13, 2021 Research Use Only CDC Flu SC2 Multiplex Assay Primers and Probes at https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex-primer-probes.html
7. CDC SOP#: DSR-052-05 PREPARATION OF VIRAL TRANSPORT MEDIUM at https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf
8. Biswas B. (2016). Clinical Performance Evaluation of Molecular Diagnostic Tests. *The Journal of molecular diagnostics*: JMD, 18(6), 803–812. https://doi.org/10.1016/j.jmoldx.2016.06.008
9. Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm071287.pdf


# 15. Summary of changes

05/10/2022 V02: Component cap color change

# 16. Symbols Displayed On The Label

| | |
|---|---|
|  | Lot number, identifies the reagent batch |
|  | Product code, identifies the IVD medical device |
|  www.omixon.com | Consult electronic Instructions for Use |
|  | Contains sufficient reagents for <N> tests. The number accompained with this symbol Indicates the total number of tests that can be performed with the IVD medical device |
|  | Manufacturer, the date accompained with this symbol refers to the date of manufacture |
|  | Storage temperature, indicates the temperature limits to which the medical device can be safely exposed |
|  | Expiry date |
|  | In Vitro Diagnostic medical device |
|  | The European Conformity (en) or Conformité Européenne (fr) mark indicates compliance with 98/79/EC European Directive on in vitro diagnostic medical devices. |

# 17. Contact Information and Support

For general assistance with this protocol, please contact:

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