Sensor Protocol updated V.2

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[1]Atom Bioworks

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1 Works for me dx.doi.org/10.17504/protocols.io.bqarmsd6

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ABSTRACT

The CoV-SARS-2 Antigen Diagnostic kit is a test intended for the qualitative detection of intact SARS-CoV-2 virions (SARS-CoV-2 is abbreviated as SARS-2 herein) in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals for COVID-19 diagnosis.

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GUIDELINES

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

Collecting the specimen:

- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV)
- Follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 1-3 ml of appropriate transport media, such as viral transport media.

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation.
- Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.
- Store specimens at 2-8 ^{O}C and ship overnight to CDC on ice pack. If a specimen is frozen at -70 ^{O}C or lower, ship overnight to CDC on dry ice.

Storing Specimens

- Specimens can be stored at 2-8 ^{O}C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70 ^{O}C or lower.

MATERIALS TEXT

CONTENTS OF THE KIT

- PCR tubes with DNA-STAR™ Assay solution
- 50 mL 1x Tris-Acetate Buffer
- 100mL Negative Control
- 100mL Positive Control
- Instructions 'For Use' manual
- AND1100 Fluorometer
- Adapter for AND fluorometer
- 25 Optically Clear PCR Tubes

Other parts you will need to start:

- Gloves,
- Eppendorf tubes,
- 10uL and 200uL pipette and pipette tubes,
- Eppendorf stand,
- ABW DNA star Assay kit,
- marker

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SAFETY WARNINGS

- Dispose off hazardous or biologically contaminated materials according to the practices of your institution.
- All patient specimens and positive controls should be considered potentially infectious and handled accordingly. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).

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BEFORE STARTING

Blank, Positive and Negative Controls:

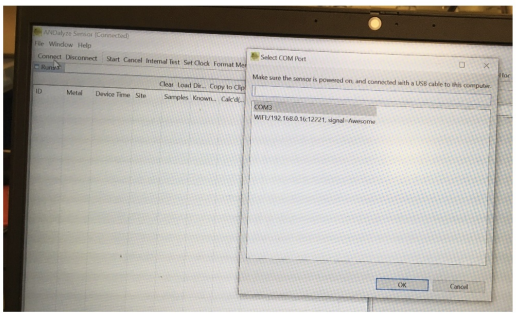
Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and that the test is correctly performed. DNA STAR COVID-19 Assay kits contain a Positive Control and a Negative Control. These controls will monitor the entire assay. Test them once with each new shipment received and once for each untrained operator. Further controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, or your lab's standard Quality

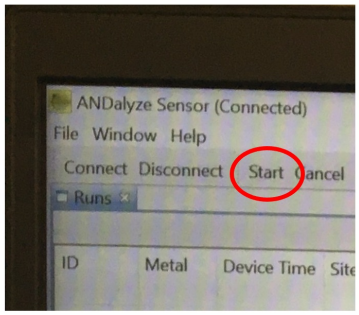
Control Test Procedures:

- Materials Required for positive control: buffer, 1 PCR tube with DNA star assay, Positive control provided in the DNA star kit
- Materials Required for negative control: buffer, 1 PCR tube with DNA star assay, Negative control provided in the DNA star kit
- Materials required for Blank: buffer, 1 PCR tube with DNA star assay, Virus sample matrix

How to use AND1100 Fluorometer

1. Connect the AND1100 fluorometer to the computer
2. Turn on the device by pressing ON/OFF
3. Start the ANDalyze software program by clicking on the AND icon
4. Connect the Fluorometer to the software by clicking 'Connect' and selecting the COM port the USB is attached to and click OK. The tab will say 'Connected' once the connection is established





5. Close the cap of the Fluorometer chamber and click 'Start' either on computer or on AND fluorometer
6. Once the reading is done. The result will show up on the runs tab as shown below.
7. To extract the results, select the run id you want to save and select 'Copy to clipboard' function as highlighted in the picture below

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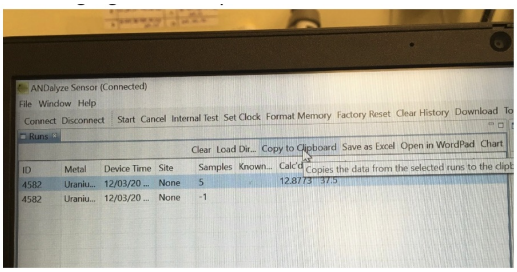
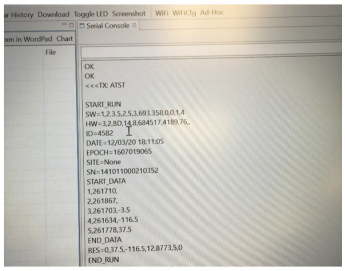




Preparation of tests 3m

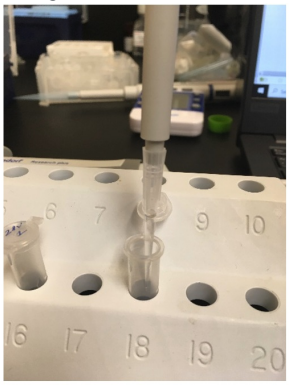
1 Prepare the following three groups of tests

- **Blank:** Prepare a 100uL of blank solution by adding 10uL of the virus matrix (Matrix in which the samples to be tested are stored; can be PBS/Saliva/Water etc.) in 90uL of the buffer
- **Positive and Negative controls**: Prepare a 100uL of positive control/negative control solution by adding 10uL of the positive /negative control provided in the kit in 90uL of the buffer provided in the kit
- **Test Sample:** Prepare a 100uL of test sample by adding 10uL of patient sample in 90uL of the buffer provided in the kit

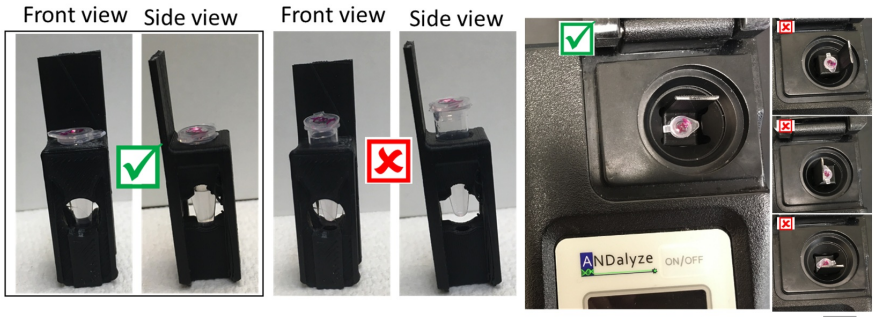
Run positive and negative control 30m

2 Before each batch of sample testing, it is recommended to run positive control and negative control to ensure the DNA Star sensor is functional. 30m

- Incubate the PCR test for 20 min (every test contains 20uL of the DNA start assay solution)
- Set up the AND1100 fluorometer for test (refer to description on how to run test on fluorometer)
- Add 80uL of the blank/positive/negative solution to the PCR tube containing DNA star assay and start the timer



- Quickly mix the solution gently by rotating the PCR tube in and up and down motion with your hand. DO NOT VORTEX OR MIX WITH PIPETTE
- To record the fluorescence, insert the PCR tube into the provided Fluorometer device adapter, make sure the tube is securely positioned so that the tube cannot move



- Close the cap of the chamber and record the fluorescence reading after 30 seconds and 10m of incubation time

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- Save your data in Excel (described in How to use Fluorometer section) and analyze data (described in data interpretation section) to ensure (Vpos-Vneg)/Vneg>15%. If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Atom Bioworks Technical Support during normal business hours before testing patient specimens.

Test the sample 10m

3 10m

- Repeat steps in last control test but using Test sample (Refer to Preparation of tests for steps to prepare sample), and get fluorescence reading after 30 seconds and 10 min of incubation time.

Results and data interpretation 1m

4 1m

For testing the sensor and fluorometer using negative control and positive control

1. Calculate an average for the five values collected per run at 30sec and 10m
2. Record the difference in the average fluorescence reading at 10m from 30sec for negative sample this is the Vneg value.
3. Record the difference in the average fluorescence reading at 10m from 30sec for positive sample, this is the Vpositive value.
4. Calculate the % increase in fluorescence signal intensity from Vneg to Vpos. If the % change is greater than 15%, proceed to evaluation of patient samples. If the difference is <15%, repeat steps 1 through 10. If the difference is <15% again, contact Atom Bioworks help@atombioworks.com

For test samples:

1. Calculate an average for the five values collected per run at 30sec and 10m
2. Record the difference in the average fluorescence reading at 10m from 30sec for Blank, this is the Vcontrol value.
3. Record the difference in the average fluorescence reading at 10m from 30sec for test sample, this is the Vsample value.
4. Calculate the background subtracted fluorescence count for the patient sample using the formula (((Vsample – Vcontrol)/ Vcontrol)*100). If the % change is greater than 15 % then the sample is positive for intact SARS-CoV-2 virions.

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