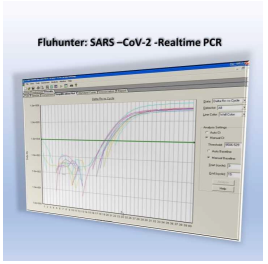
May 31, 2024

Detection of SARS CoV-2 in different samples with a singleplex and mutation resistant real time PCR assay



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We use this protocol and it's working

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Disclaimer

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Abstract

Genekam has developed an unique ready to use PCR kit for the detection of SARS CoV-2 indifferent samples. This was developed on 20.01.2020 just at the beginning of the pandemic outbreak. This test is mutation resistant test, hence mutations taking place in different part of this virus is not effecting the performance against many tests available in literature and market, which cannot provide reliable results because the mutations affect them strongly. This test can be used to find the performance of other tests being used in different laboratories in the world because old samples can be tested with this kit only as mutations have no effect. This is reliable and stable along with good sensitivity. Many approved kits from different manufactures were withdrawn from the market as well as some of protocols provided through world organizations have to be modified because of the mutations, but not Genekam FR754 ready to use real time PCR.

Protocols provided from different organisations used 3 different genes of the virus, therefore many users are using a multiplex assay. It is well known that multiplex assay lack the sensitivity as achieved with singleplex test. Our test is a singleplex assay, that does not have the quality problems as faced through such multiplex assay. This targets only one conversative part.

This test is highly specific also along with using on RNA of broader range of samples like buccal and nasal swab, urine, cell culture, lymph nodes, plasma, respiratory tract samples, other body fluids and tissue samples. This is tested with related and non-related targets like RSV, Influenza A strains (H1N1, H3N2, H5N1), Influenza B, CMV, EBV, Salmonella, Chlamydia, Maesles, Staphylococcus aureus, Yellow fever, Zikavirus, Mumps, Rubella, Herpes Simplex Virus 1&2, various healthy human samples ec. These are a few examples.

The test is in position to detect the Wuhan strain and its mutated versions like South African mutations (B.1351 and B 1.1.529), UK mutation (B1.1.7), Brazil mutation (P1), Indian mutation (B1.617), Omicron mutations (B.A.2.86, XBB 1.5 like, XAK), B.1.427, C37 and B.1621 as examples. This shows that this test is mutation resistant!

Sensitivity: 0.000003pg/µl, (0.003fg/µl)

Image Attribution

Fluhunter SARS-CoV-2 Kit

Guidelines

The results will be shown as Ct values.

Materials

FR475 - Fluhunter: SARS CoV-2 (Realtime PCR-Kit) CE
-UDI-DI: 04262420430676
optional:
FR799 - Identification of Human specific ß-Actin -Internal Control CE
-UDI-DI: 04262420430010

Compositions of the Kit:
- Tube A (2 tubes): 2 x 385µl
- Tube B (2 tubes): 2 x 550µl
- Tube Y (1 tube): 110µl
- Positive (+Ve) Control (tube D1) (1 tube): 40µl
- Negative (-Ve) Control (tube D2) (1 tube): 60µl

Equipment needed:
- Realtime PCR thermocycler
- Laboratory centrifuge
- 96 well microplates for PCR or Microtubes (0.2ml)
- Sterile Pipette-tips with filter (1-10µl & 10-100µl)
- Pipettes (quality pipettes)
- Vortexer
- RNA isolation kit: Genekam Biotechnology AG, Germany or equivalent

Safety warnings

- It is only for in vitro use.
- It must be used through trained persons.
- It should not be used after expiry date.
- During PCR, one should work very cleanly.
- All works must be done in different parts of laboratory.
- NEVER STORE THIS KIT WITH FOOD, VACCINE OR BACTERAL CULTURES.
- Decontaminate the instruments regularly (once a week).
- To dispose of the kit and its contents, add 70% ethanol to the contents of the kit and then dispose of it.

Before start

1. User must read the manual before use.

2. Important Hint:
Real time PCR is based on fluorogenic dyes. In this kit, the probe is labeled with the following dyes: namely Carboxy-fluorescein (reporter called FAM) and 6- Carboxy tetramethyl rhodamine (quencher; called TAM). The results will be shown as Ct values. Up to a Ct value of 40, the samples should be considered positive. Ct values between 40 and 45 should be considered marginally positive (doubtful).

1 Thaw **one tube** each: A, B, Y, D1 and D2. After thawing, put the tubes at 4°C (as it is better). If the kit
is not in use, store them at -20°C.

2 Mark your microtubes with a sample number, +ve Control and –ve Control. Otherwise use a 96 well microplate instead of tubes.

3 Add 7µl of tube A to each tube.

4 Add 10µl of B to each micro tube. Avoid touching the wall of the microtubes.

5 Add 1µl of Y to each tube (Do not touch the wall).

Important Hint:
User can calculate the total requirement of chemicals needed. User can mix 7µl of A + 10µl of B + 1µl of Y together in one tube for one reaction, but to have 10 reactions, there will be total volume of 180µl (70µl Tube A, 100µl B and 10µl Y). From this, 18µl can be distributed into each tube. This step saves time and hardware.

6 Add 2µl of your RNA template (RNA isolated from samples) with sterile filter pipette-tip into each microtube according to your labelling, except +Ve and -Ve (Do not touch the wall). **Use a new pipette tip for each sample**!

7 Use a new pipette tip with filter. Add 2µl of tube D1. This is the positive control supplied with the kit.

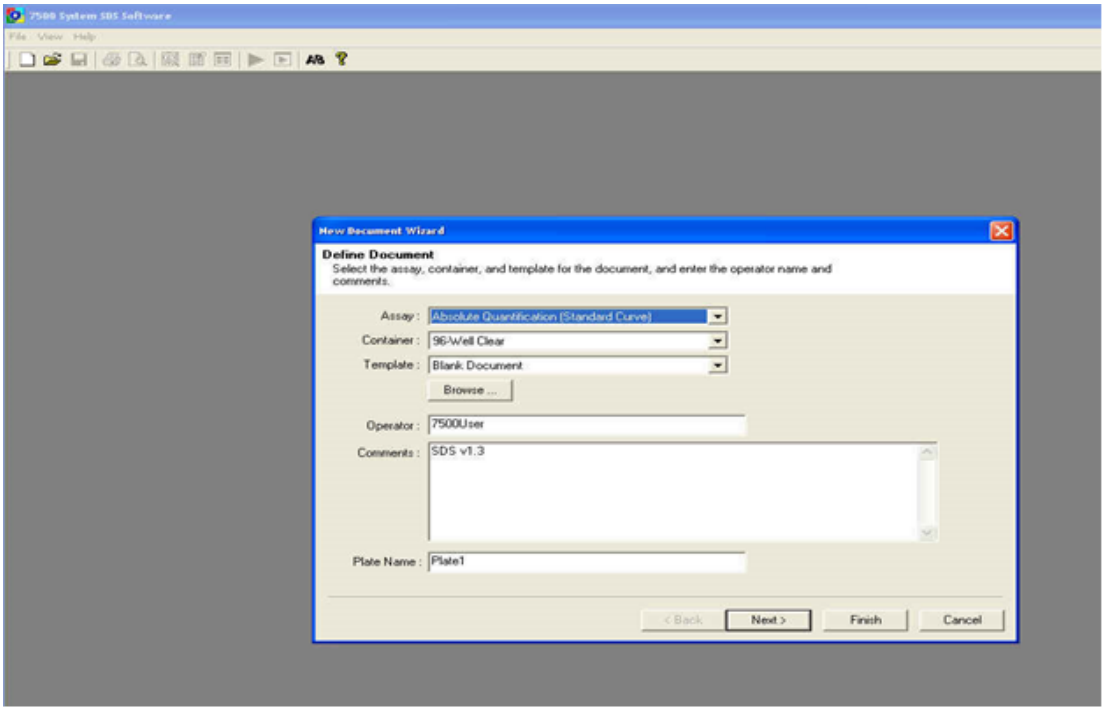
8 Use a new pipette tip. Add 2µl of -Ve (tube D2) to -Ve Control (Do not touch the wall).

9 Run the program of your thermocycler as follows: Check if everything is added correctly, as the volume of each microtube or well must be almost the same.
Now enter quencher and reporter dye to setup your software (see FAQ) and run the following program (total time needed: 2h, 30m)

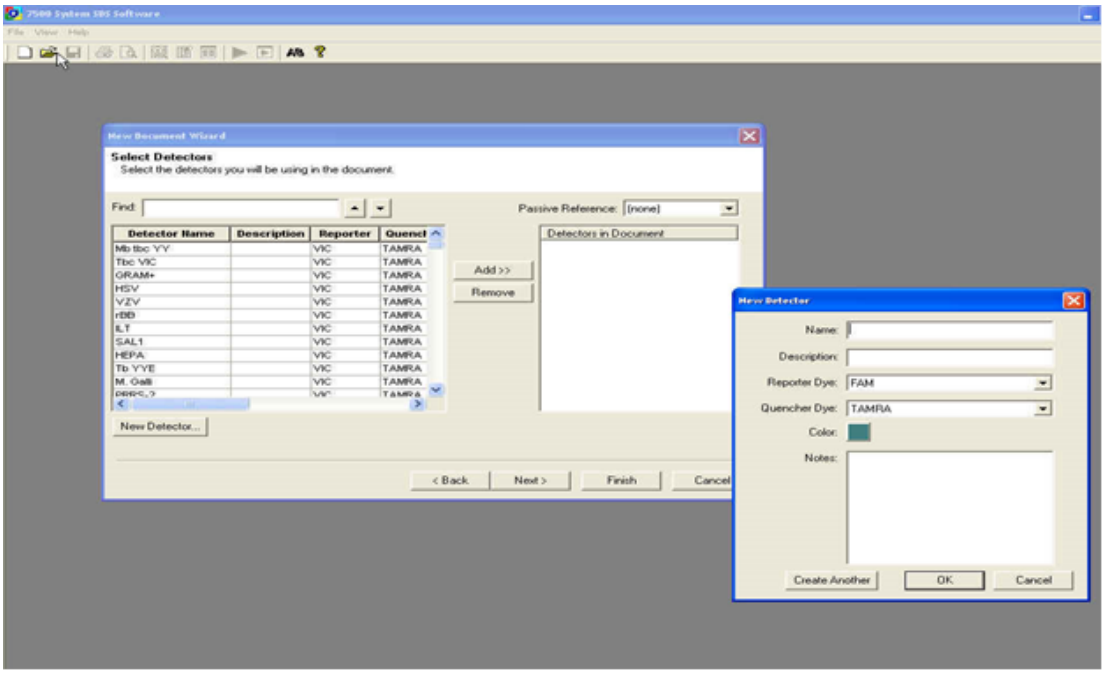
1. 60 minutes at 42°C
   10 minutes at 70°C
   15 seconds at 95°C x 45 cycles
   60 seconds at 60°C

10 **Example of setting up a real time machine:**
Instructions for real time PCR (RT-PCR System: ABI 7500)

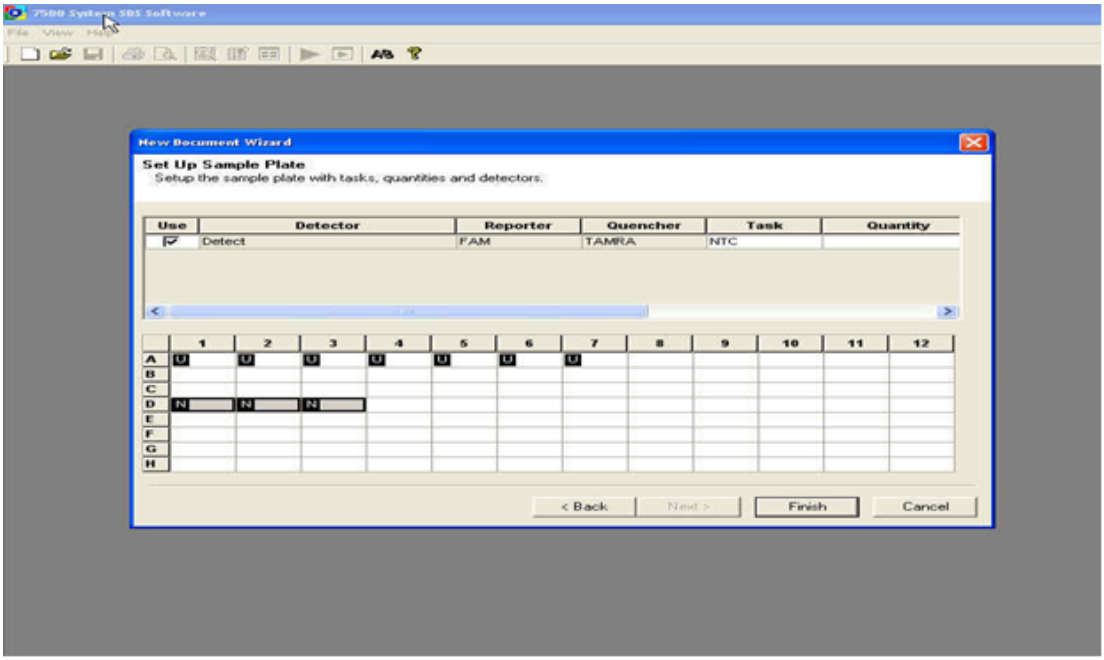
Open SDS Software - **File New** - Select: Absolute Quantification assay - Write name - Click: **Next**



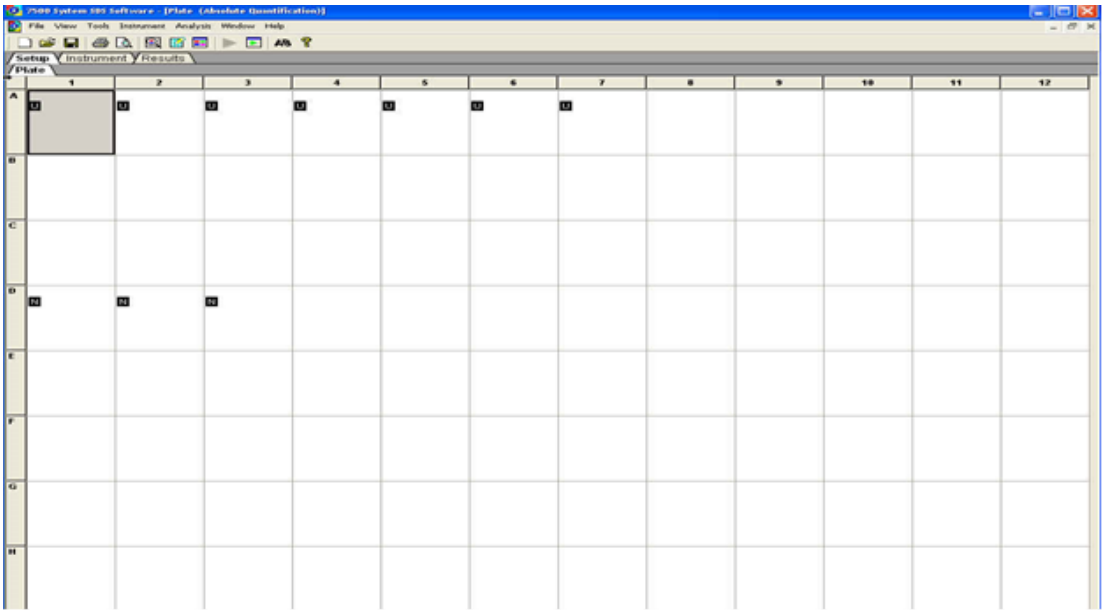
11 Create a **New Detector**: Select **Reporter** and **Quencher**-Dye: (FAM-TAMRA) - Click: Add - Select: Passive Reference-none - Click: **Next**



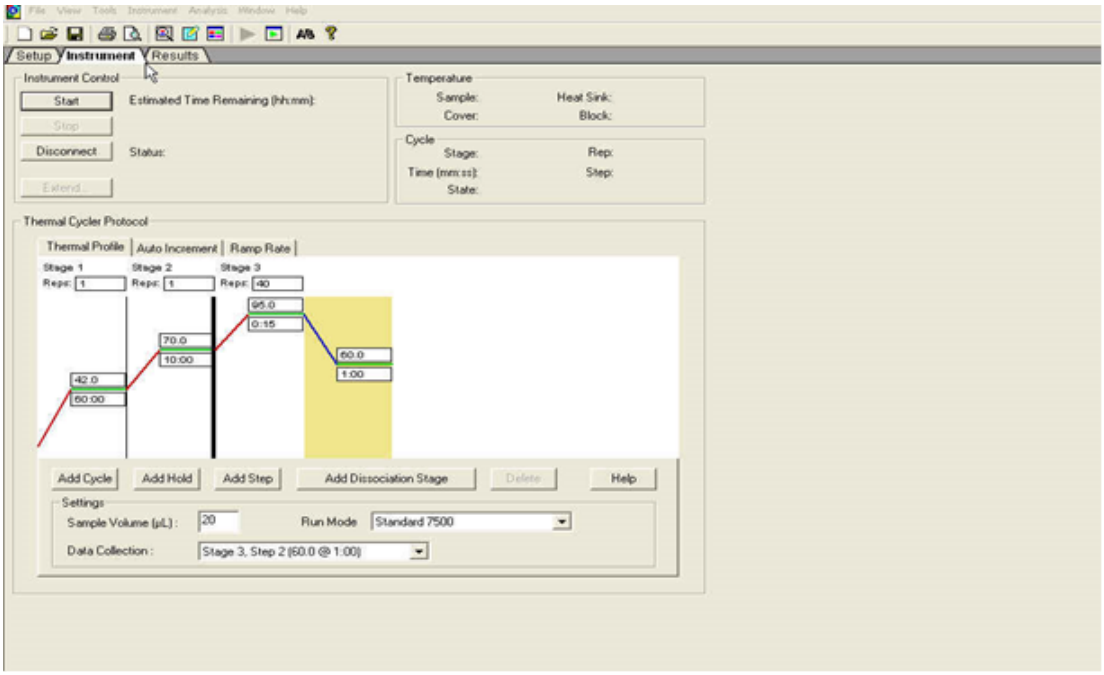
12 Select Well positions: **U**nknown - or –**NTC** (No Template Control) - or -**Standard** - Click: **Finish:** The Plate Document will open.



13 Write the name of samples for each well or tube.



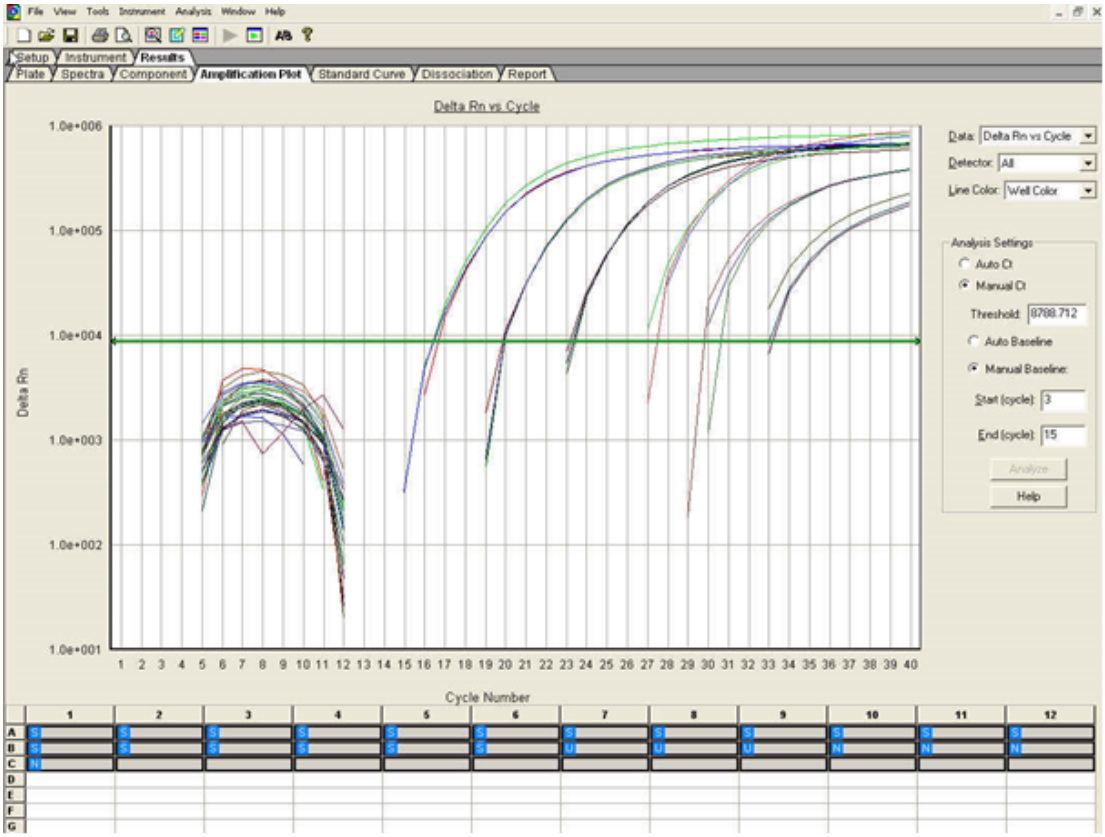
14 Select Instrument Tab - Insert the **Program** to the thermal Profile **as mentioned in point 10** - Change sample Volume (20µl) - Click: File - **"Save as"** to save the Setup - Click: **"Start"** to begin the PCR run: Shortly the run time will be displayed on the screen.



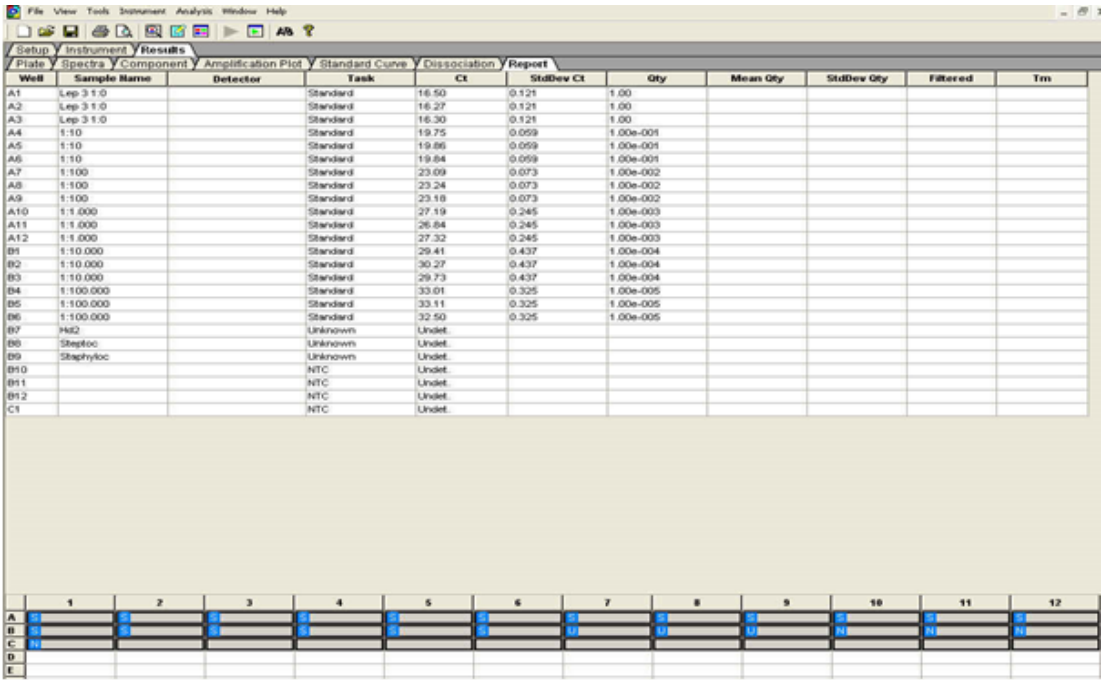
Before starting the PCR program, check that the plate or tubes are properly sealed. **The wells of the well plate or microtubes must be in contact with metal block** (important!). There should be no air or lose contact with metal block of thermocycler. In case of 96-well plate, it should be sealed with adhesive cover. Now run your PCR.
Once the PCR is finished, a small dialog box will appear: “The run has been completed successfully” Click: OK. Now you can take out the microtubes or well plate.

15 **STEP B**

Click the **Result**-Tab and the **Amplification**-Tab: Place the Threshold line above the background, then select: “Analyse” (see Figure below). Calculate the threshold cycle (Ct) for each well. There should be no signal in the negative control. Successful positive controls or positive samples must give a curve in the software graphics.



16 The Report page provides the Ct-values and Standard deviation for each well.



Protocol references

Bhatia S. Pitfalls in the performance of real time PCR tests for SARS CoV-2 and time to improve these tests. Microbes Infect Dis 2023; 4(4): 1079-1080. DOI: **10.21608/mid.2023.239655.1630**