



PCR STATION PROCESS FLOW

Last updated: 10 Oct 2020

Release: API v1.4

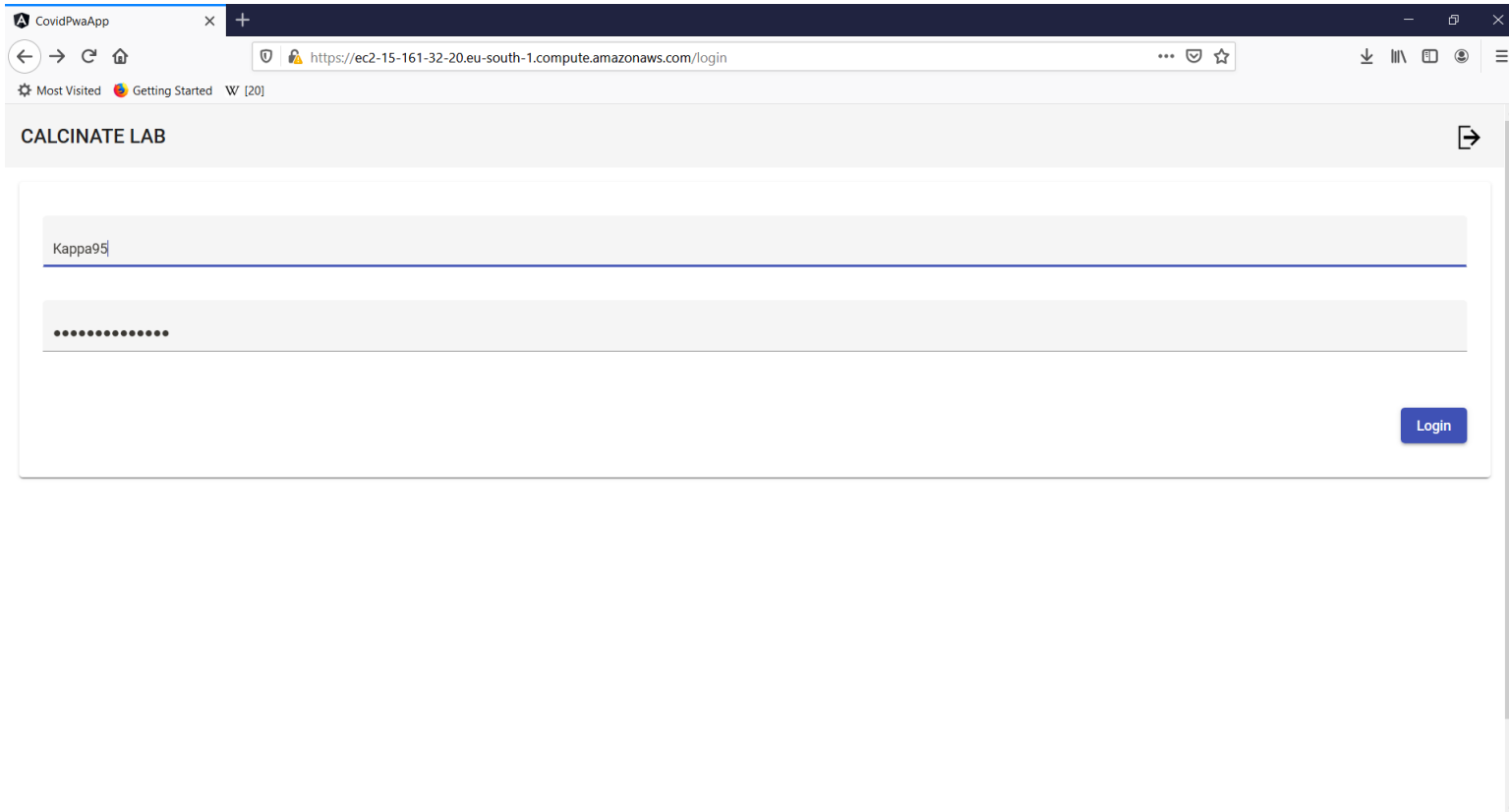
This is a process flow and instructions document for the PCR station of the COVID-19 testing Lab at Ospedale F.M. Passi, developed under the Covmatic project

This Process flow document is valid for the **BioRad API version 1.4**

ALWAYS Print this document in A4 Landscape format to ensure good readability

In case you just started the system (computer), open the Covmatic digital interface webpage with the link provided:
<https://ec2-15-161-32-20.eu-south-1.compute.amazonaws.com/login>

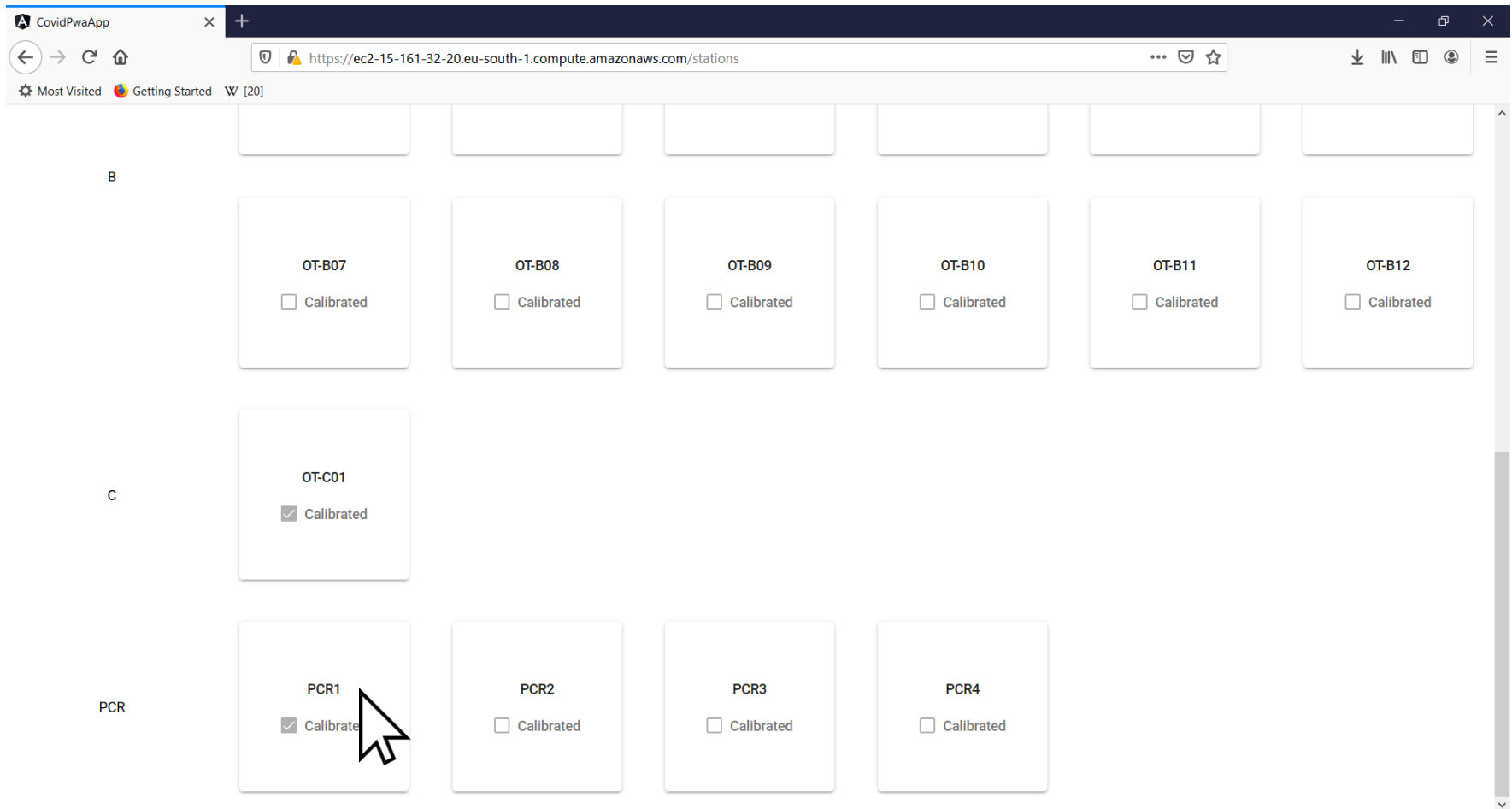
Enter your credentials and Login

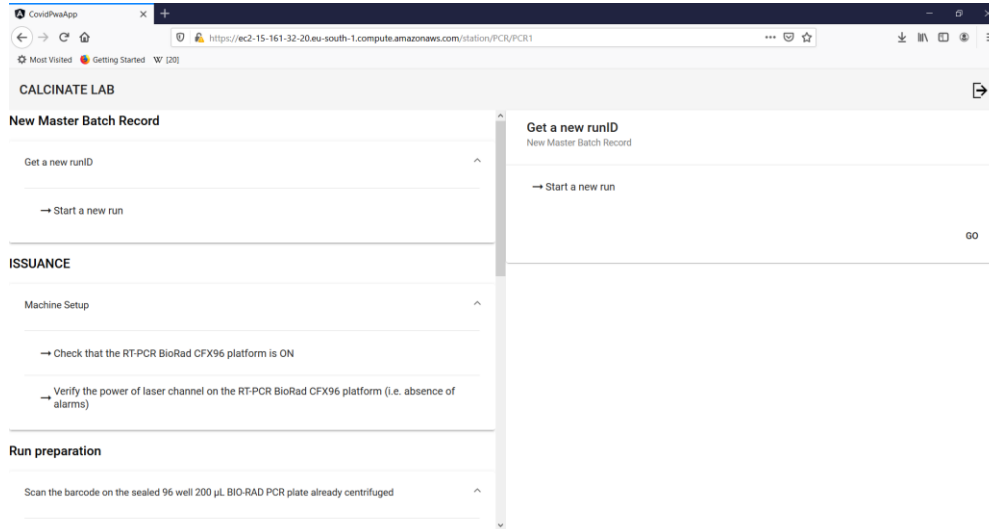


The screenshot shows a web browser window with the title 'CovidPwaApp'. The address bar displays the URL <https://ec2-15-161-32-20.eu-south-1.compute.amazonaws.com/login>. The browser's bookmark bar shows 'Most Visited', 'Getting Started', and 'W [20]'. The main content area has a header 'CALCINATE LAB' with a right-pointing arrow icon. Below the header is a login form with two input fields: the first contains 'Kappa95' and the second is masked with dots. A blue 'Login' button is positioned at the bottom right of the form.

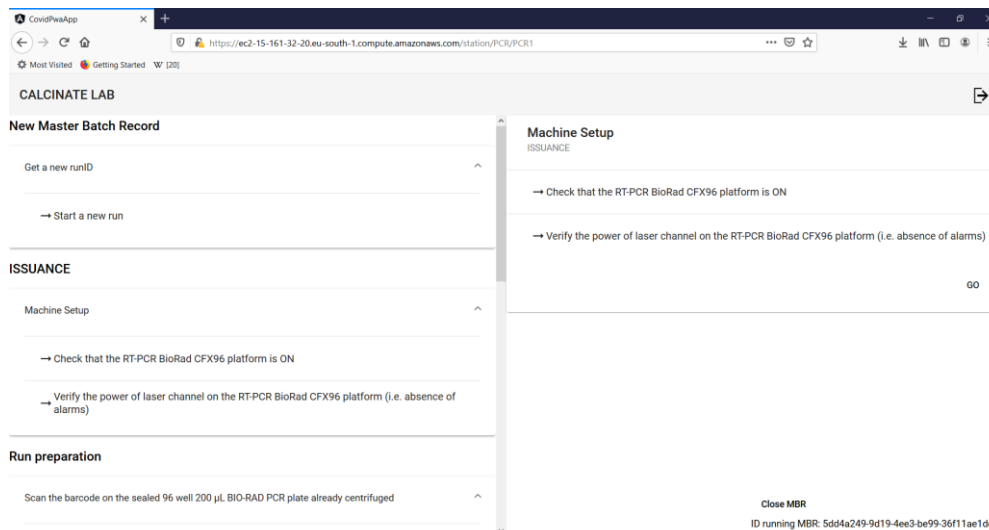
Remember to use a face mask and hand gloves!

Scroll down and select the number of the PCR station that you're working on. Please **BE CAREFUL** while making this selection. Select that PCR number on which you are working:

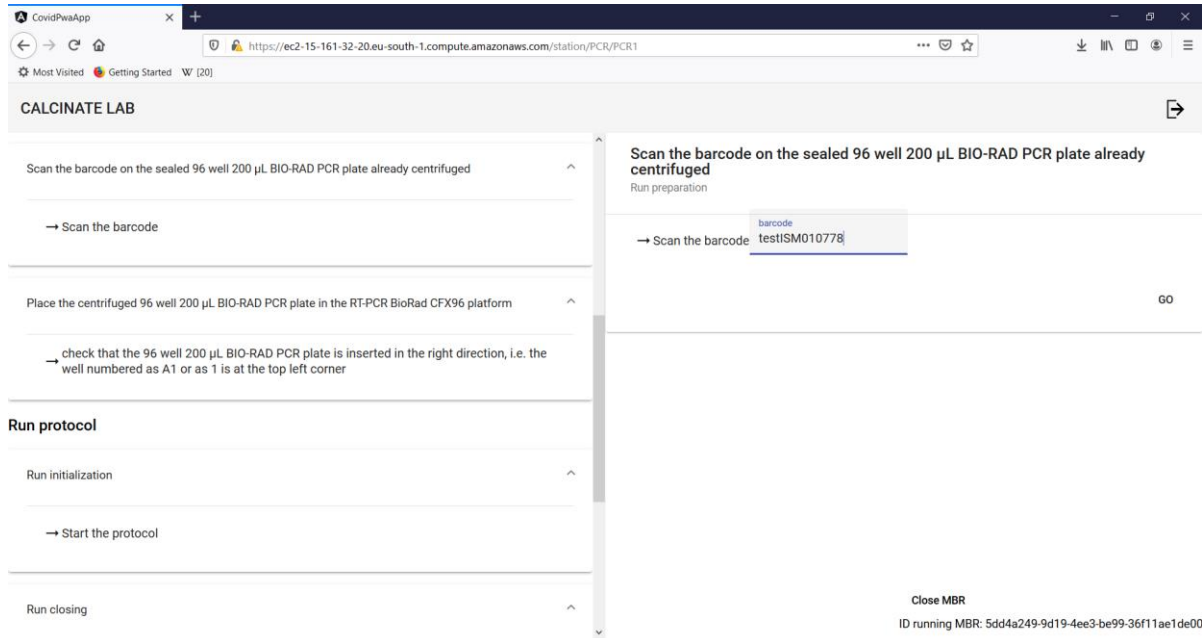




Step 1: Make sure that you have the PCR well plate and it is ready to be processed. Click “Go” on this page



Step 2: Check that the PCR is connected to the computer (with USB serial) and it is switched ON and in IDLE state. Make sure that there are no alarms. Click “Go” on this page.



CALCINATE LAB

Scan the barcode on the sealed 96 well 200 µL BIO-RAD PCR plate already centrifuged

→ Scan the barcode

Place the centrifuged 96 well 200 µL BIO-RAD PCR plate in the RT-PCR BioRad CFX96 platform

→ check that the 96 well 200 µL BIO-RAD PCR plate is inserted in the right direction, i.e. the well numbered as A1 or as 1 is at the top left corner

Run protocol

Run initialization

→ Start the protocol

Run closing

Scan the barcode on the sealed 96 well 200 µL BIO-RAD PCR plate already centrifuged

Run preparation

→ Scan the barcode

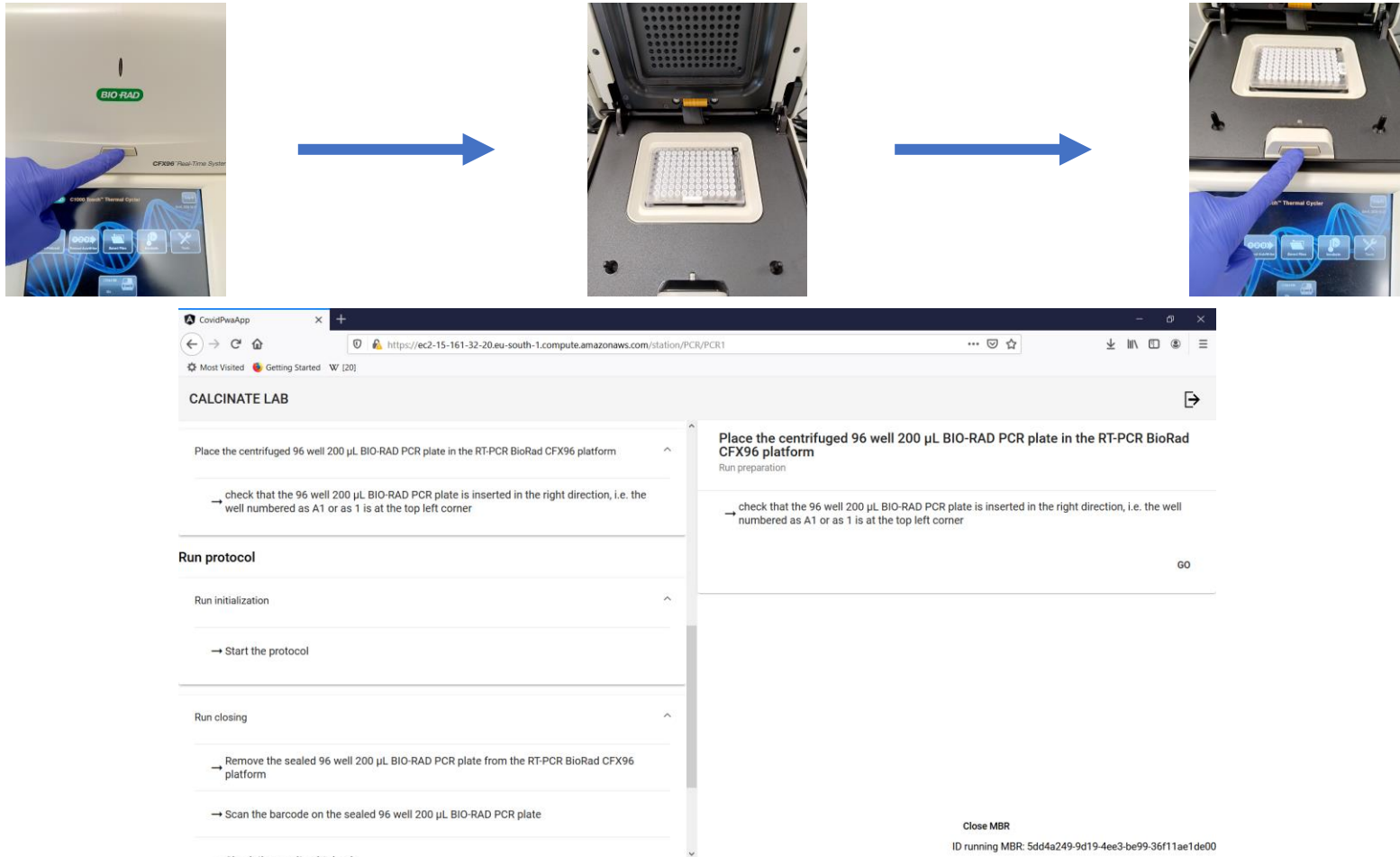
GO

Close MBR

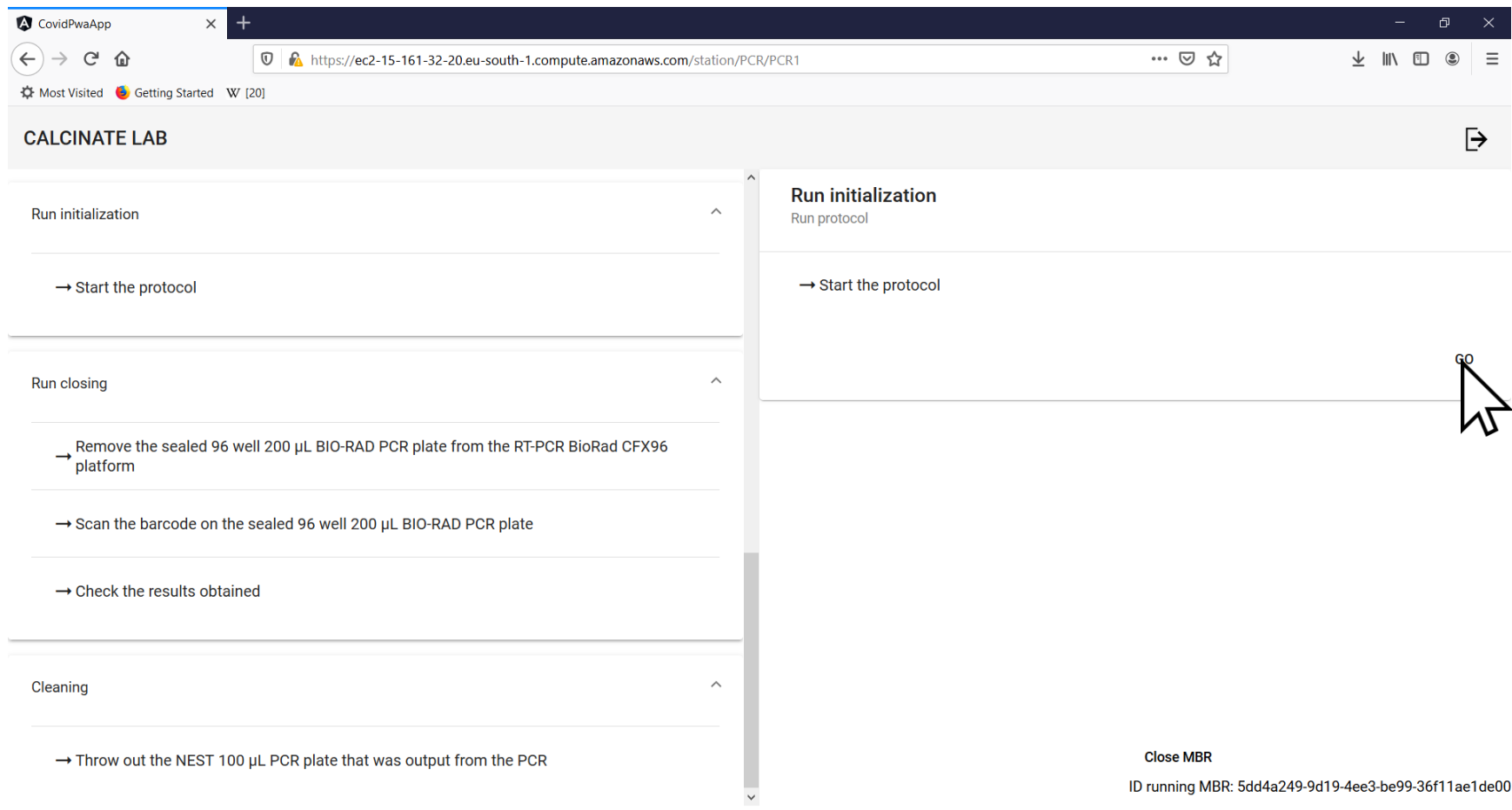
ID running MBR: 5dd4a249-9d19-4ee3-be99-36f11ae1de00



Step 3: Scan the Barcode on the PCR well plate. Make sure that the Barcode is scanned **ONLY ONCE**. Click “Go” on this page once the barcode is scanned



Step 4: Open the PCR module lid by pressing the button, insert the PCR well plate in the correct position (as shown), and press the button again to close the PCR lid. Click on “Go” AFTER completing this procedure



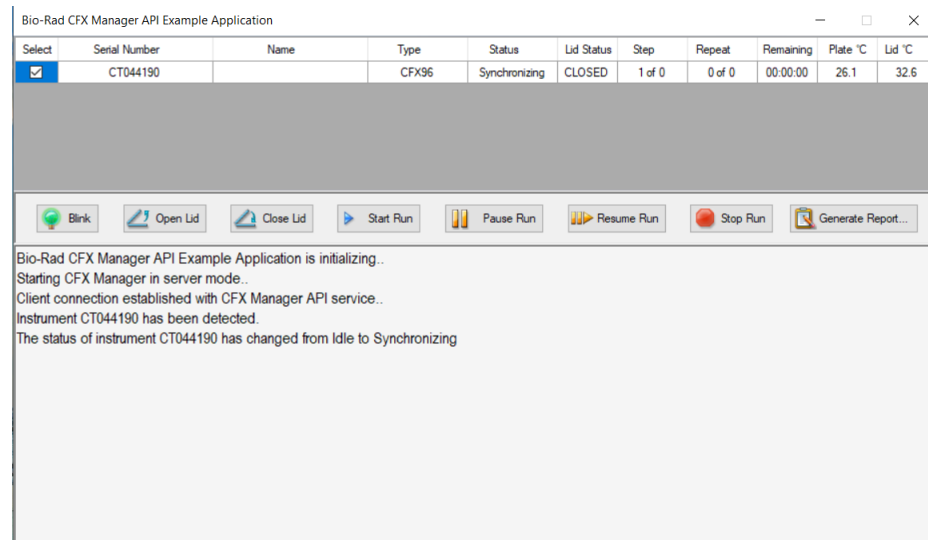
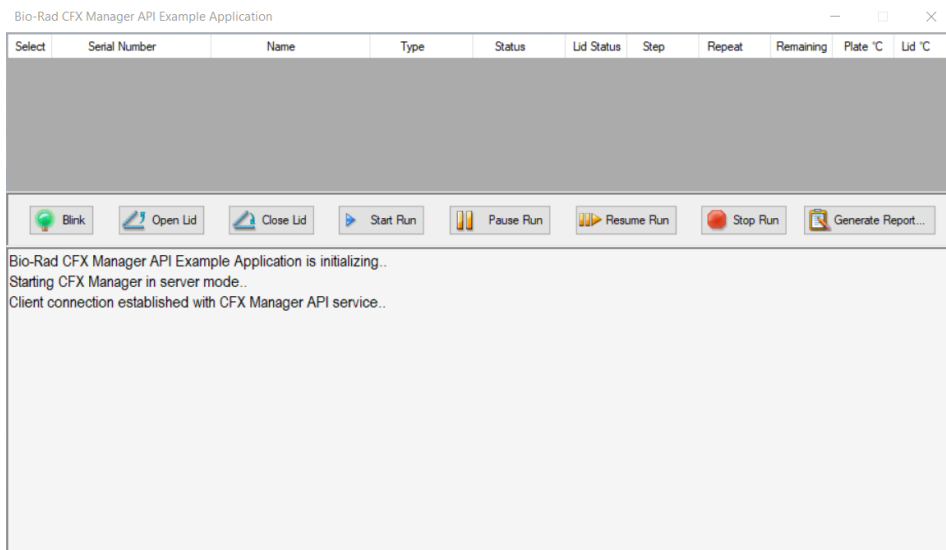
The screenshot shows a web browser window with the address bar displaying `https://ec2-15-161-32-20.eu-south-1.compute.amazonaws.com/station/PCR/PCR1`. The page title is 'CovidPwaApp'. The main content area is titled 'CALCINATE LAB' and contains a list of steps for a PCR protocol. A mouse cursor is pointing at a 'GO' button on the right side of the 'Run initialization' step.

CALCINATE LAB

- Run initialization
 - Start the protocol
- Run closing
 - Remove the sealed 96 well 200 μ L BIO-RAD PCR plate from the RT-PCR BioRad CFX96 platform
 - Scan the barcode on the sealed 96 well 200 μ L BIO-RAD PCR plate
 - Check the results obtained
- Cleaning
 - Throw out the NEST 100 μ L PCR plate that was output from the PCR

Close MBR
ID running MBR: 5dd4a249-9d19-4ee3-be99-36f11ae1de00


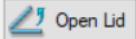
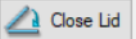
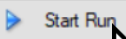
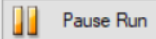
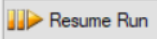

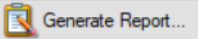
Step 5: AFTER the lid is CLOSED, make sure that there are no applications of CFX Manager or CFX Maestro or BioRad API running on the computer. Close these applications and wait for 15-20 seconds if they are running. THIS IS CRUCIAL, OTHERWISE THE API WILL NOT LAUNCH. Click on “Go” to start the Protocol



Step 6: The BioRad API will launch automatically (as shown above). Allow some time for the API to detect the instrument. **DO NOT CLICK ON ANY BUTTONS IN THE API BEFORE THE INSTRUMENT IS DETECTED.** Once the instrument is detected, the API will display it as shown above.

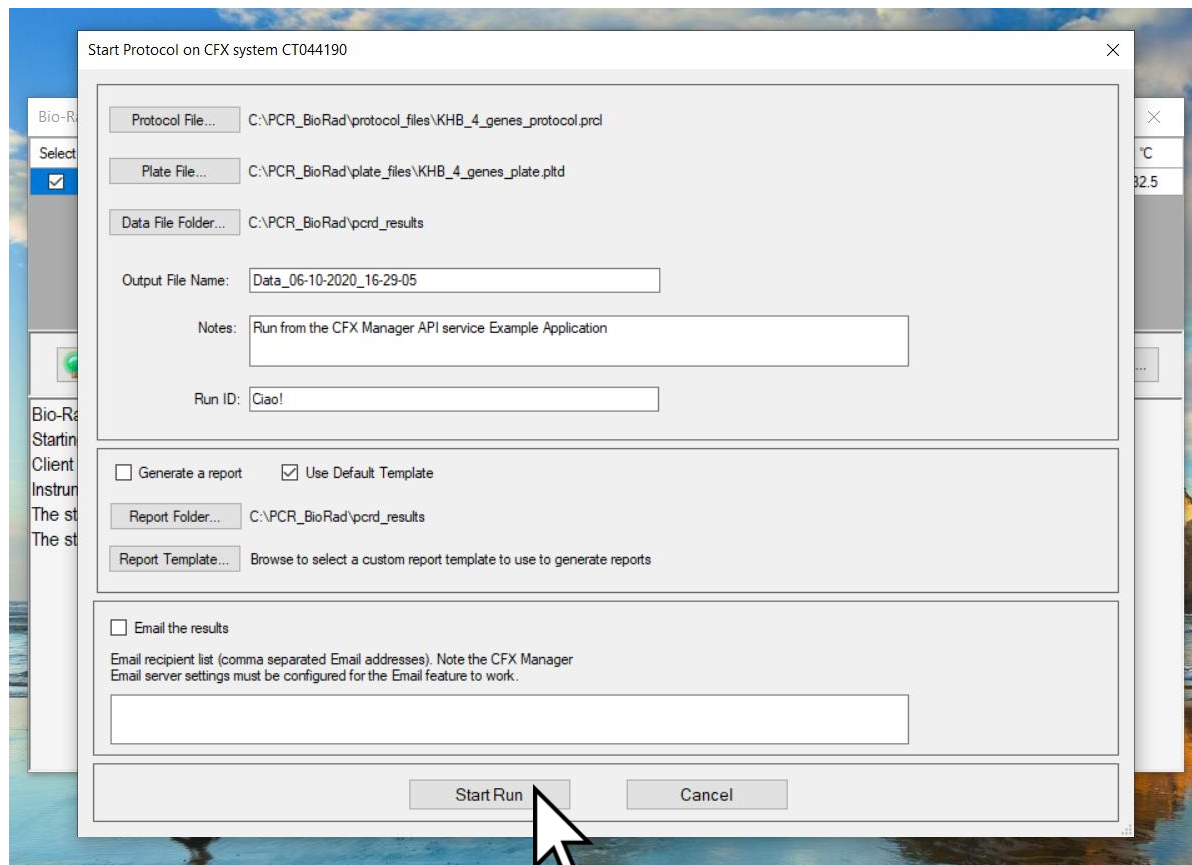
Bio-Rad CFX Manager API Example Application

Select	Serial Number	Name	Type	Status	Lid Status	Step	Repeat	Remaining	Plate °C	Lid °C
<input checked="" type="checkbox"/>	CT044190		CFX96	Synchronizing	CLOSED	1 of 0	0 of 0	00:00:00	26.1	32.6

 Blink
  Open Lid
  Close Lid
  Start Run
  Pause Run
  Resume Run
  Stop Run
  Generate Report...

Bio-Rad CFX Manager API Example Application is initializing..
 Starting CFX Manager in server mode..
 Client connection established with CFX Manager API service..
 Instrument CT044190 has been detected.
 The status of instrument CT044190 has changed from Idle to Synchronizing


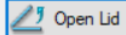
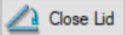
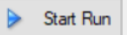



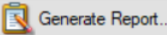
Step 7: Once the Status of the instrument changes from “Synchronizing” to “Idle”, click on “**Start Run**”. A new window will open



Step 8: Make sure that the correct Protocol file, Plate file and Data File Folder are selected. In this case, they are selected by default. Click on **“Start Run”**. The PCR will initialize and then the protocol will start.

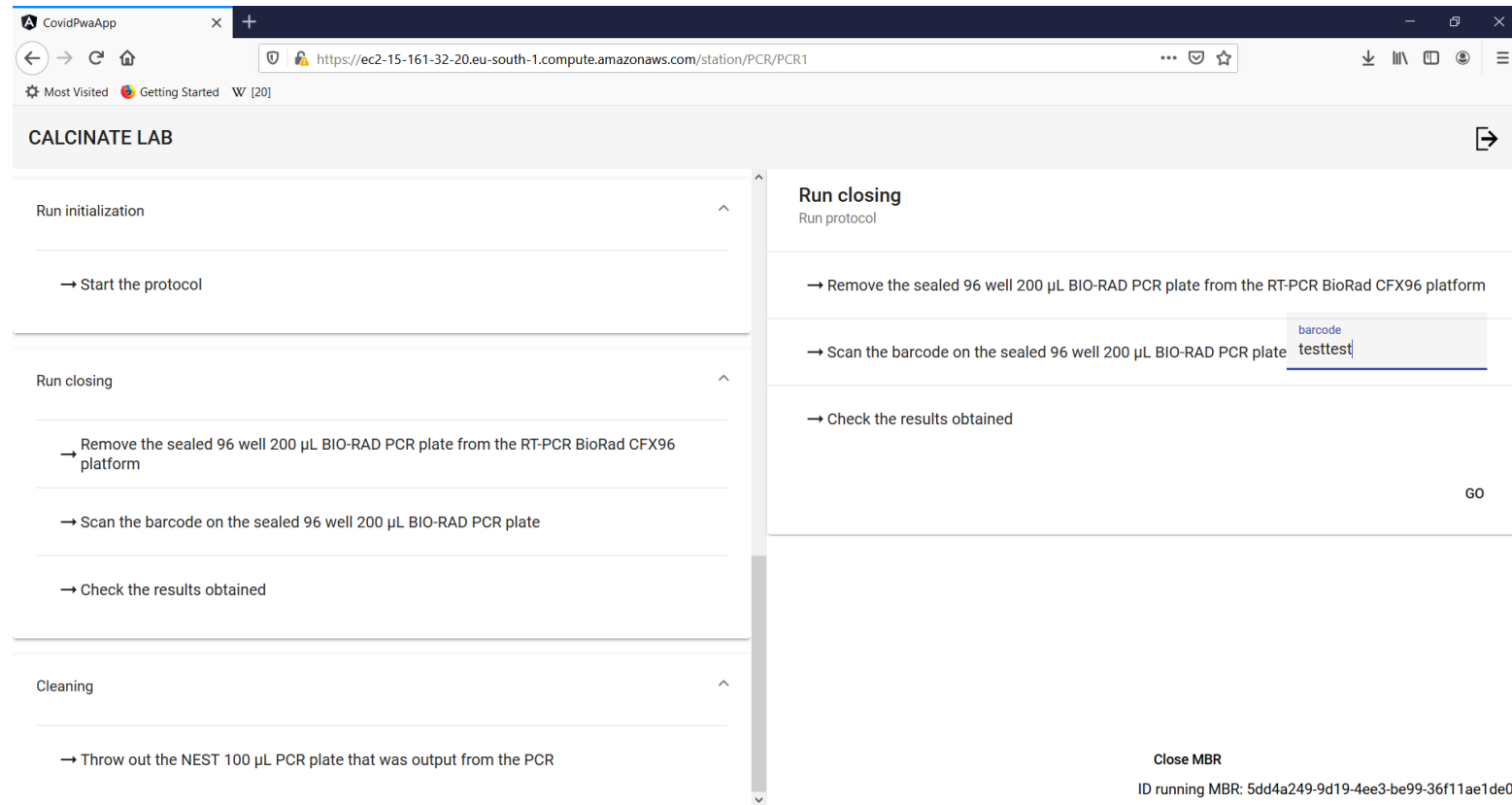
Bio-Rad CFX Manager API Example Application

Select	Serial Number	Name	Type	Status	Lid Status	Step	Repeat	Remaining	Plate °C	Lid °C
<input checked="" type="checkbox"/>	CT044190		CFX96	Processing	CLOSED	1 of 9	0 of 0	00:00:00	58.4	102.9

 Blink
  Open Lid
  Close Lid
  Start Run
  Pause Run
  Resume Run
  Stop Run
  Generate Report...

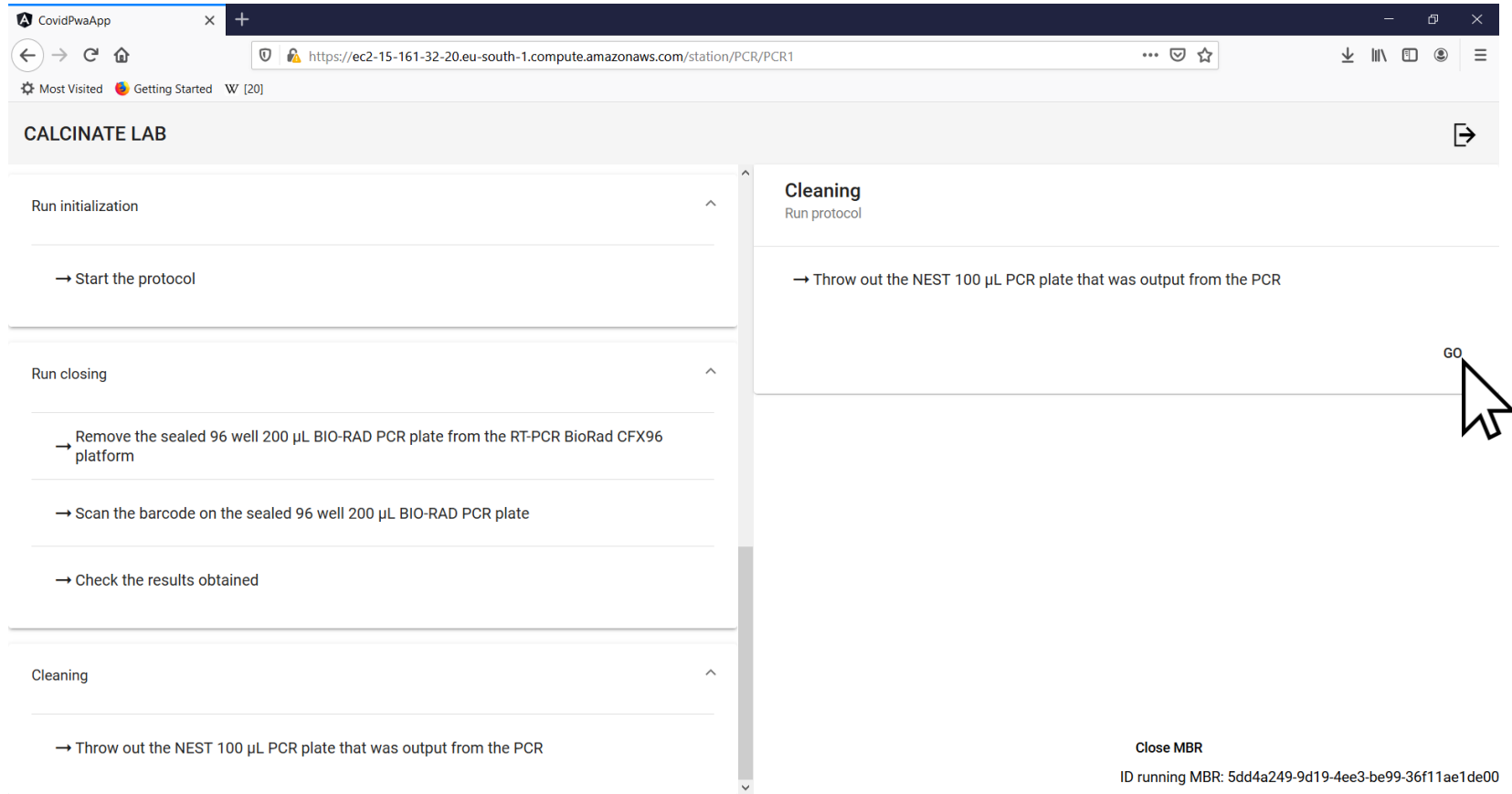
Bio-Rad CFX Manager API Example Application is initializing..
 Starting CFX Manager in server mode..
 Client connection established with CFX Manager API service..
 Instrument CT044190 has been detected.
 The status of instrument CT044190 has changed from Idle to Synchronizing
 The status of instrument CT044190 has changed from Synchronizing to Idle
 Sending RunProtocol to CFX system CT044190...
 Instrument CT044190 is initializing.
 A protocol has begun running on instrument CT044190
 A protocol has finished running on instrument CT044190, which is currently processing the data.
 Instrument CT044190 has completed processing the data from a completed protocol run.

Step 9: Once the Protocol run is complete, allow some time for the API to process the data. DO NOT CLOSE THE API APPLICATION MANUALLY. The **API will close automatically** once it has processed the data



Step 10: Go back to the Covmatic digital interface webpage. **Remove the well plate from the PCR** and scan its barcode again (take care! The PCR lid gets very hot during the protocol run). Click on "Go" after scanning barcode

To check the results of the run, go to "C:\PCR_BioRad\pcrd_results" folder and open the most recent .pcrd file
Alternatively, you can also access the .pcrd results by going to "OneDrive\Desktop\BIO RAD\TEST RESULTS" and selecting the correct PCR folder



The screenshot shows a web browser window with the address bar displaying `https://ec2-15-161-32-20.eu-south-1.compute.amazonaws.com/station/PCR/PCR1`. The page title is "CovidPwaApp". Below the browser window, the interface is titled "CALCINATE LAB" with a right-pointing arrow icon. The main content area is divided into two columns. The left column contains three sections: "Run initialization" with a sub-section "→ Start the protocol"; "Run closing" with sub-sections "→ Remove the sealed 96 well 200 µL BIO-RAD PCR plate from the RT-PCR BioRad CFX96 platform", "→ Scan the barcode on the sealed 96 well 200 µL BIO-RAD PCR plate", and "→ Check the results obtained"; and "Cleaning" with a sub-section "→ Throw out the NEST 100 µL PCR plate that was output from the PCR". The right column is titled "Cleaning" with a sub-section "Run protocol" and a sub-section "→ Throw out the NEST 100 µL PCR plate that was output from the PCR". A mouse cursor is pointing at a "GO" button in the right column. At the bottom right, there is a "Close MBR" button and a text string "ID running MBR: 5dd4a249-9d19-4ee3-be99-36f11ae1de00".

Step 11: Throw away the used well plate safely and Click “Go” on this page. The protocol run is complete, and the data is saved in the digital server and on the local computer. The PCR is now ready for the next run!