REF Kimera P-IV

RT-PCR User Guide

Document: 94-000001-A

A product of NEXLESS HEALTHCARE

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Made in Canada

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Nexless Plasmonic, Nexless Plasmonic P-RT-PCR, Kimera are the brand names Nexless Healthcare own range of PCR Plasmonic Thermal cycler products and accessories. For further details please visit our web site.
www.nexlesshealthcare.com

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Thank You

Nexless Healthcare would like to thank you for buying Nexless Plasmonic P-RT-PCR thermal cycler. A portable real time plasmonic PCR its main application is for the detection of SARS-CoV-2 but can be used for forensics, water quality testing, bacteria, and pathogen detection applications.

The advantages of the plasmonic Nexless Plasmonic P-RT-PCR thermal cycling are as follows:

Test results can be obtained in less than few minutes

Uses simple proven modern plasmonic technology

Much improved power efficiency thus can be battery operated

Real time fluorescence amplicon monitoring

Compatible with all existing PCR methodology

Sterilization achievable by extended plasmonic heating

Reaction volume from nanoliters to microliters

Intended Use

The Nexless Plasmonic P-RT-PCR system is a portable real time plasmonic PCR for converting, amplifying, and detecting DNA and RNA targets. The plasmonic feature of this PCR device makes it very fast and very sensitive for quantification of nucleic acids in specimens. The main application of this PCR device is to detect SARS-CoV-2 very accurately and extremely fast. It can be used on other applications such as forensics, water quality testing, genetically modified organism detection, gene analysis, cancer phenotype, and bacteria and pathogen detection.

The Nexless Plasmonic P-RT-PCR system results can be used for diagnostic procedures.

The Nexless Plasmonic P-RT-PCR system is intended for indoor use.

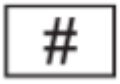
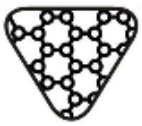
Table of Contents

Table of Figures

Safety Warning

 WARNING	Indicates Risk of Radiation Exposure. Could, if not avoided, result in serve injury or death. Class 4 is the highest and most dangerous class of laser. It can burn the skin, or cause devastating and permanent eye damage as a result of direct, diffuse or indirect beam viewing.
	Indicates Risk of biological Hazard.
 DANGER	Indicates Risk of Electric Shock Hazard, which could, if not avoided, result in serve injury or death. Proceed with appropriate caution.
 DANGER	Indicates a Burn Hazard which could, if not avoided, result in serve injury or death. Proceed with appropriate caution.
 DANGER	Indicates a Risk of Explosion which could, if not avoided, result in serve injury or death. Proceed with appropriate caution.
 WARNING	Indicates a hazardous situation which could, if not avoided, result in serve injury or death; or severely damage the unit.
 CAUTION	Indicates a hazardous situation which could, if not avoided, result in minor or moderate injury; or degrade or impair the functionality of the unit. To indicate that caution is necessary when operating the device or control close to where the symbol is placed, or to indicate that the current situation needs operator awareness or operator action to avoid undesirable consequences.
	Consult instructions for use.
	Keep dry.
	Fragile. Handle with care. This medical device can be broken or damaged if not handled carefully.

Symbols

	Nexless Healthcare, Mount Royal, Quebec, Canada www.nexlesshealthcare.com
	Made in Canada
	Date of manufacture
	Authorized representative in the European Community
 YYYY-MM-DD	“Use by” date
	Lot number, Batch code
	Catalog number: Kimera P-IV
	Model number: 49-500001-D
	Serial Number
	Temperature limit, Lower limit and Upper limit
	This equipment is intended to be used as an in vitro detection or in vitro diagnostic medical device.
	Indicates a control material that is intended to verify the results in the expected negative range.
	Indicates a control material that is intended to verify the results in the expected positive range.
	Indicates the total number of IVD tests that can be performed with the IVD medical device.
	The reagents to use with this medical device must be combined with gold nano particles.
	The reagents are for single use only. Do not reuse.

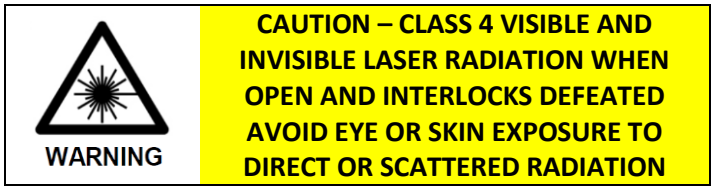
Safety Precautions

Important Precautions

- The protection provided by this equipment may be impaired if it is not used in a manner described in this manual.
- It is essential that the user of this equipment is aware of the potential hazards associated with the unit and its accessories.
- All operators should be familiar with the safety precautions and warnings given in these instructions before attempting to operate the unit.
- Improper use of this unit or its accessories may impair their functionality and invalidate the manufacturer's warranty.

Laser related precautions

- This product contains four lasers that are Class 4, the most dangerous class of lasers.



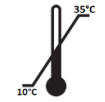
- Lasers have a maximum optical power of 4W and operate at 808nm.
- Lasers are enclosed inside the product and are operational only when the top lid is closed.
- Magnetic safety switch will shut off the lasers if the lid is open.
- Do not override the magnetic safety switch as this could expose the user and the surroundings to direct or scattered radiation.
- This product complies with IEC 60825-1, 3rd Edition, May 2014.

Unit Handling Precautions

- Care should be taken not to drop the unit or subject it to rough physical handling, both during normal use and during transportation and storage.
- The unit should be supported using the base of the unit when being lifted or moved. Do not lift the unit by any other part of the casework. Turn off the power before lifting or moving the unit.
- Care should be taken when lifting the unit due to its weight.
- Care should be taken not to knock the LCD display.
- Do not use excessive force when touching the touch screen or operating the buttons.

Unit Installation and Operating Environments

- The unit is designed for indoor laboratory use only.
- The acceptable operating temperature range is 10°C to 35°C



- The acceptable operating relative humidity range is 20% to 80% non-condensing.

- If the unit is stored in conditions outside of these ranges, it must be left to stand without power until it has acclimatized to within these environmental limits before being powered.
- Use only the AC mains power cord provided with the unit.
- The unit must be connected to a suitably earthed mains supply, with appropriate earth-leakage and over-current protection.
- Always ensure that the mains power connector is securely inserted into the rear of the unit, and any excess power cord does not pose a potential trip or pull hazard.
- The power for this unit must come from a stable mains supply. Do not power from inverters, frequency convertors, or generators.
- This unit requires a power supply with both a stable voltage and stable frequency. Inverters, frequency convertors, and generators do not provide acceptable stability and the unit can perform outside its specification, or fail to perform, or be damaged by the use of these forms of power supply.
- Do not operate the unit in any area which is, or has been, or is thought to have been exposed to explosive or flammable gases, vapors, or liquids.
- The unit must be installed and operated on a solid, stable, and level working surface; ensuring that the ventilation holes under the unit and at the rear of the unit are not obstructed.

General Operating Precautions

- Ensure that the power is switched off at both the AC mains supply outlet and at the back of the unit before inserting or removing the mains power cord.
- The unit can reach temperatures of over 120°C and can remain of for a time after the unit system is turned off.
- If conditions are set such that the experiment is finished with the plate at a high temperature it will exit the unit with the potential to burn the operator and care should be taken.
- The unit is intended for use with plates containing biological samples only with appropriate reagents and gold nanoparticles.

- Reagents with gold nanoparticles are intended to be used only once. Do not reuse reagents.
- Never use the unit to seal any explosive, volatile or highly reactive substances or chemicals.
- There is a possible finger crush hazard due to the moving parts of the door. Care should be taken when closing the door.
- Do not block ventilation at the front and back of the instrument. Leave at least six inches of space on all sides of the instrument when powered on.
- Wear protective eyewear, clothing, and gloves when handling reagents or operating the instrument.

Unit Maintenance and Serviceability

- There are no user or operator serviceable parts inside the unit. Do not remove the unit casework.
- Servicing should be performed by authorised Nexless service center.
- Any attempt to open and or modify the device will void the manufacturer’s warranty.
- Removal of the unit's casework will void the manufacturer’s warranty and may expose the user to a Risk of Electric Shock resulting in serious injury or death.
- Once installed, the externally accessible unit fuse will only blow under a fault condition. This fuse should only be changed after the unit has been thoroughly inspected by a qualified engineer.
- Always switch off the unit and disconnect the power cord before performing any cleaning or decontamination procedure.
- If liquid is spilt into or over the unit, switch off and disconnect the power from the AC mains outlet before attempting to deal with the spillage.
- Ensure that the unit has cooled down to room temperature before performing any cleaning operation and before moving or storing the unit.
- The use of harsh chemicals and cleaning agents may damage the unit and degrade its performance.
- Always follow the cleaning and decontamination procedures as specified in this instruction manual.
- Do not autoclave any part of the unit or its accessories.

Marks of Conformity

FC	This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation. This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.
CE	This device complies with the Electromagnetic Compatibility Directive (2014/30/EU) and Low Voltage Directive (2014/35/EU) of the European Union. This device complies with the Regulation (EU) 2017/746 of the European parliament and of the council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. This device complies with the Medical Devices Directive 93/42/EEC.
	Marking of electrical and electronic equipment in accordance with article 11(2) of Directive 2002/96/EC (WEEE). Waste Electrical and Electronic Equipment Directive 2012/19/EU. Do not dispose of the instrument with general waste
RoHS	Based on the information provided by our supply lines, this device is RoHS complaint according to the Directive 2011/65/EU, Directive 2015/863 and Directive 2017/2102/EU
REACH	Nexless monitors Substances of very high concern (SVHC) that are candidates to be listed in Annex XIV of REACH.

Technical Specifications

General	
Plasmonic PCR 4 wells	P-RT-PCR-P-IV
Weight	4.3 lbs
Dimensions	9” (23cm) Wide x 9” (23cm) Deep x 7” (18cm) High
Compliance	CE, UL
Reaction Volumes	20 µl
Sample format	4-tube strips 200 µl
Runtime	Less than 15 min for 3-step 40 cycles PCR
Electrical	
Power voltage	100 to 240VAC
Frequency	50 or 60 Hz
Max power	60 W
Networking	Ethernet, USB
Touchscreen	7.0 inch, capacitive
Operating temperature	10 to 35°C
Relative humidity	20 to 80% non-condensing
Hardware	
Thermal cycling system	Plasmonic based, 4 wells
Plasmonic laser	808nm VCSEL
Max ramp rate heating	5.5°C/sec
Average ramp rate cooling	5°C/sec
Programmable temperature range	45 to 100°C
Temperature accuracy	±0.5°C
Lid Temperature	Ambient to 110°C
Excitation	Individual Blue LEDs
Measurement	Individual measurements
Detection	Individual Photo Diodes
Software	
Operating system	Linux Embedded
Memory	Up to 100 tests
Run mode	Touchscreen program selection Multi menu configuration for easy use Online fluorescence display
Customisation/Programming	Programmable Electrical Medical System
Connected to PC	PC monitoring, analyzing tools and programming
Disposables	
4-Tube Strips	4-Tube Strips, clear. Includes caps
Reagents	4-Well PC tube strip prefilled with reagents
Gold nano rods	Gold Nanorods solution ready to use
Swabs	Nasopharyngeal swabs & universal media

Reagents	
DNA Stain	SYTO™ 16 Green Fluorescent Nucleic Acid Stain
Polymerase	Taq Polymerase
Reverse transcriptase	Protoscript II
	RNase Inhibitor
Plasmonic agent	Gold Nano Rods (GNR)
	d2H2O
Run Mode	
Standalone	Touchscreen support for instrument configuration Preprogrammed experiments execution Flexible experiment programming and execution Online fluorescence display
Connected to PC	PC monitoring, analyzing tools and programming
Connected to LAN	Support of online monitoring using LAN connection Support of remote Nexless Service Software upgrade Support of AI
External devices	Support of temperature calibration kit via USB Support of external barcode scanner via USB Support of external printer via USB
Instrument active communication	Web notification, with success or failure messaging and optional experiment file attachment
Applications	
Licensed for real-time PCR	Yes
Range of excitation / emission	488/509 nm
Detection formats	Intercalating dyes

Background Information

Plasmonic PCR

Infectious pathogens remain a serious threat to the health of all individuals of all age groups across the globe. This has been compounded by the emergence of antimicrobial resistance adding to the severity of pathogenic infections.

Infections can also cause serious damage to other important activities like the food and diary industry, agricultural and forestry which can lead to severe consequences for the people and industries affected. For all the above reasons it is imperative that microbiological identification need to be accomplished much faster than is currently the standard. The recent COVID pandemic highlights the need for rapid detection as classical infection management procedures are failing. To address these needs, a Point-of-care or point-of-infection diagnostics is clearly required as to allow health profession or others in related fields to confer near instantaneous diagnoses that will allow proper infection control measures and the choice of antibiotics if need be.

Rapid testing to identify pathogens method used will have a dramatic affect on morbidity and mortality and reduce health costs such as length of hospital stay and medication costs. Our Canadian economy relies heavily on agriculture, food, meat, and diary products all vulnerable to infections which can incur very high costs.

McGill University (Drs. Andrew Kirk, Miltiadis Paliouras and Mark Trifiro) at Montreal's Jewish General Hospital has developed a light and nanotechnology driven, Plasmonic PCR technology. Such technology has introduced the capability of very rapid PCR testing. It also allows for the development of novel instrumentation, from hand-held devices for field work to large instruments required in central laboratories. The platform uses specially prepared "nano-heaters" to drive an ultra-fast PCR reaction. The reaction can be temperature monitored and managed with exquisite control by non-contact pyrometers. An immediate infection notice is attainable with complete testing within minutes.

Polymerase Chain Reaction (PCR) is a critical tool for biological research investigators allowing for exquisite genetic identification. Plasmonic PCR, which employs the very efficient heat transfer of light-activated metallic nanoparticles, is simple and can drive PCR reactions very quickly. Future generations of plasmonic PCR technology will introduce various platforms from portable point of care diagnostic devices to large central laboratory multiplexing devices. We have now introduced a real time plasmonic PCR with the ability of ultra-fast cycling with fluorescence-monitoring of amplicon production.

PCR cycle

Kary Mullis invented polymerase chain reaction (PCR), in which a small amount of DNA can be amplified in large quantities. By applying heat, the DNA molecule's two strands are first separated, and the DNA primers then. With the help of the enzyme DNA polymerase, new DNA chains are formed, and the process can then be repeated, and the DNA can be amplified. PCR has been of major importance in both medical research and forensic science.

Each PCR cycle is composed of three stages: (1) Denaturation, (2) Annealing and (3) Elongation. At denaturation stage, at a temperature of 90 to 95°C, the DNA strands are separated into single-strand DNA. At annealing stage, at a temperature of 40 to 60°C, the primers attach to the target DNA. At the elongation stage, at a temperature of 70 to 75°C, the enzyme or DNA polymerase extends the DNA from the primers and by the same way doubles the DNA sequences. PCR cycles are repeated several times to generate thousands to millions of copies of a particular section of DNA to a detectable level.

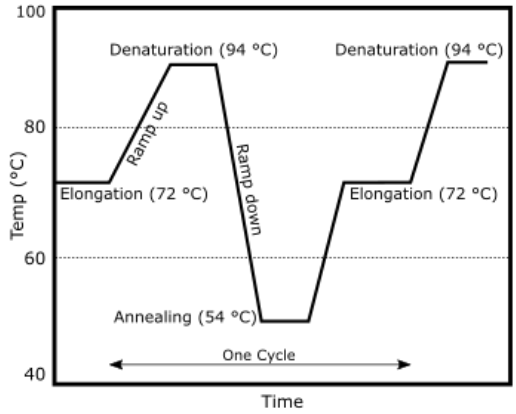


Figure 1 - PCR Temperature Cycle

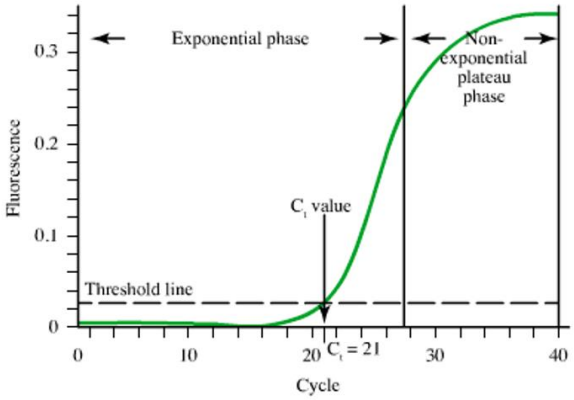


Figure 2 - PCR Amplification Plot

RT-PCR

The reverse transcript RT-PCR technique is to convert RNA into cDNA so that PCR cycles can amplify the cDNA to detectable levels.

PCR equipment

Conventional PCR equipment use heater block to heat multiple test tubes at once. Then it uses various cooling techniques to cool the test tubes at the desired temperature. The temperature cycle takes a lot of time. A Full 30 to 40 cycles process can take up to one hour and thus, cannot be used for rapid testing. The conventional PCR equipment also use a large amount of energy for heating and sometimes for cooling too.

Plasmonic PCR uses a combination of gold nanoparticles and intense lighting to generate high temperatures almost instantly, with temperature rises of up to 20 degrees per second. Cooling can be done by shutting of the light and blowing cool air to the test tubes. This results in a very fast PCR cycle, and a rapid result.

PCR applications

- Rapid detection and analysis of many products
- Medical diagnostic tester
- Forensic and DNA identity testing

Product Description

Plasmonic P-RT-PCR system

The Nexless Plasmonic P-RT-PCR system is a portable real time plasmonic PCR for converting, amplifying, and detecting DNA and RNA targets. The plasmonic feature of this PCR device makes it very fast and very sensitive for quantification of nucleic acids in specimens. The main application of this PCR device is to detect SARS-CoV -2 very accurately and very fast. But other applications such as forensics, water quality testing, genetically modified organism detection, gene analysis, cancer phenotype, and other bacteria and pathogen detection.

This PCR device is based on a plasmonic technology for heating the vials. Reagents contain gold nano particles. These particles react with an internal laser to create heat driving thermocycling. Along with cooling fans, the PCR creates a chain reaction extremely fast.

Our Plasmonic thermocycling deploys optically powered nano-heaters suspended in the PCR reaction mixture itself. When exposed to laser light, gold nanorods (GNRs) rapidly generate enormous heat in femtoseconds, making the heating cycle virtually instantaneous at rates of up to 20°C/second.

Simple, efficient, and extremely rapid thermocycling is achieved allowing for a highly specific PCR product (30 cycles in under 10 minutes for 20 µL). It is also completely compatible with all existing PCR protocols, has very convenient 10-20 µL reaction volumes, and has the potential to operate at any scale.

Nexless Plasmonic PCR technology encompasses real-time monitoring of amplicon production. In keeping with an all-optical approach, we also feature fluorescent monitoring, with Ct values indistinguishable from conventional qPCR platforms. These innovations significantly increase the scope of our Plasmonic PCR in completing and interpreting PCR in real-time.

As the energy needs for our Plasmonic PCR are vastly reduced, a self-powered portable POC version is under development.

Figure 3 - P-RT-PCR-P4 Device

RT-PCR Device

The PCR Device can accommodate up to 4 test tubes. Simply lift the top lid, insert up to 4 test tubes with the PCR ready sample to test, close the lid and run the PCR.

Features and impacts

The nanoparticle heating elements are within the sample, no heating blocks, rapid cooling.	Faster heating-cooling more reaction control
Nanoparticle bio-modifications to optimize enzyme performance.	Fidelity, Enzyme naïve, No inhibition
Highly specific, high yields.	Picomole DNA detection
Plasmonic DNA Nano-sensor read-out for detection of amplicon.	Perfect for microbial detection Low cost and rapid fabrication
Synergic development of instrument, light management, nanoparticles, and biology to produce the fastest PCR reaction.	Fastest detection in the world first in class

Plasmonic Thermal Cycling

The Gold nanorods (GNRs) convert light to heat very efficiently (>99%) and extremely fast (in femtoseconds). The laser and the GNRs have been chosen to have the maximum absorption of optical energy.

The result is a heating from the inside of the PCR reaction with an improved and uniform heating by the presence of GNRs within the reaction mixture. The GNRs are encapsulated to prevent inhibition of polymerase.

The advantages of plasmonic thermal cycling are as follows:

- Test results can be completed in less than 10 minutes
- Uses simple proven modern plasmonic technology
- Much improved power efficiency thus can be battery operated
- Real time fluorescence amplicon monitoring
- Compatible with all existing PCR methodology
- Sterilization achievable by extended plasmonic heating
- Reaction volume from nanoliters to microliters

Top lid

The top lid of the PCR-P4 features 2 knobs to adjust the heater plate to the top of the test tubes. Depending on the types of test tubes used, the heater plate needs to be adjusted using the two knobs. A level indicator slides to show the level attained.

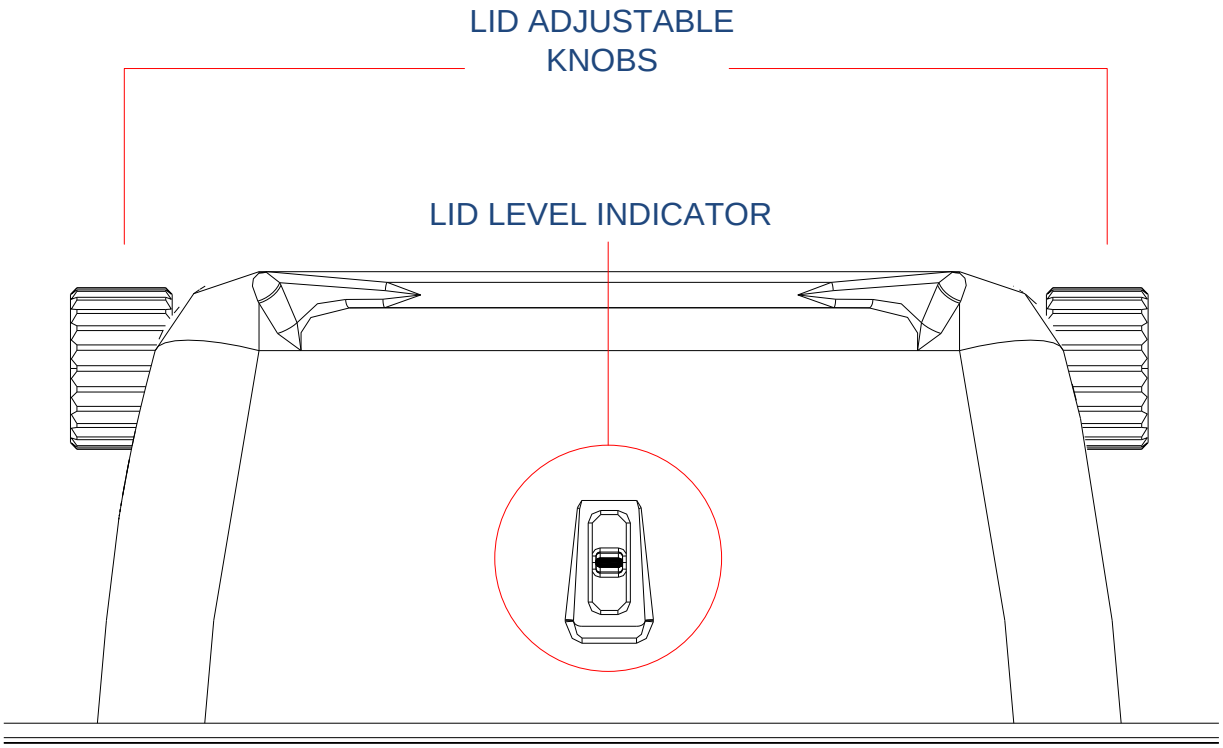


Figure 4 - View of the top lid

NOTE: As a protection, unit will not activate if lid is open. When lid is opened the Class 4 lasers are automatically shut down.

NOTE: An automatic watchdog will deactivate the unit after 2 seconds in the event of any anomaly.

NOTE: The top lid heats the top of the test tubes to a temperature that can go up to 120°C. Be careful when opening the lid as the temperature can still be hot and potentially cause burns.

Power inlet

The power inlet and the power switch are located on the rear of the device. Simply connect the power cord and, when ready, turn the switch to the ON position.

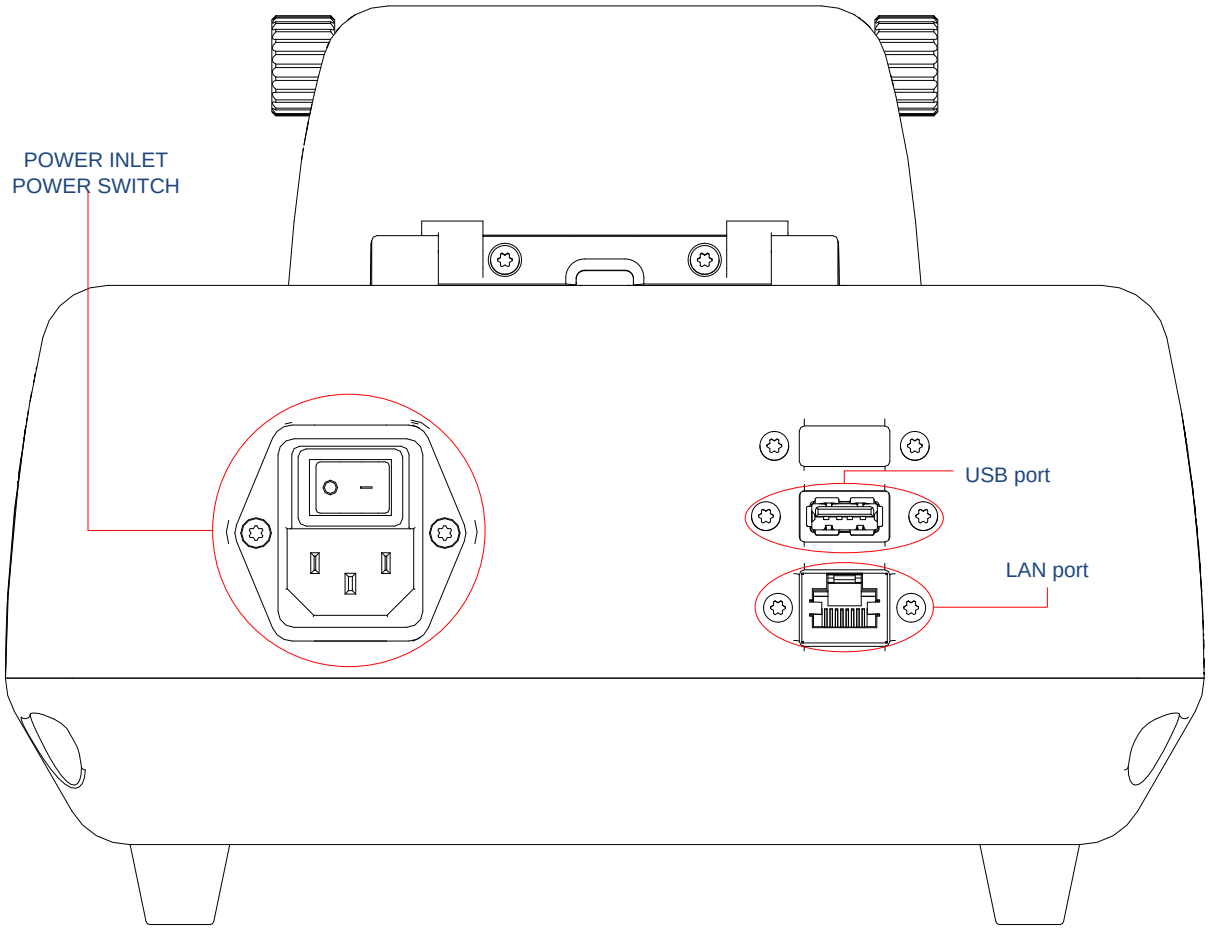


Figure 5 - View of the rear of the device

USB port and LAN port

The USB and LAN ports are located at the rear of the device. LAN port should be connected to the local network to download test reports.

USB port can be connected to a keyboard or a mouse or any other USB 2.0 interface.

Air inlet and vents

The device needs to have access to the environment air around. Air vents are conveniently located on each side on the unit and on the bottom. Make sure the vents are not obstructed.

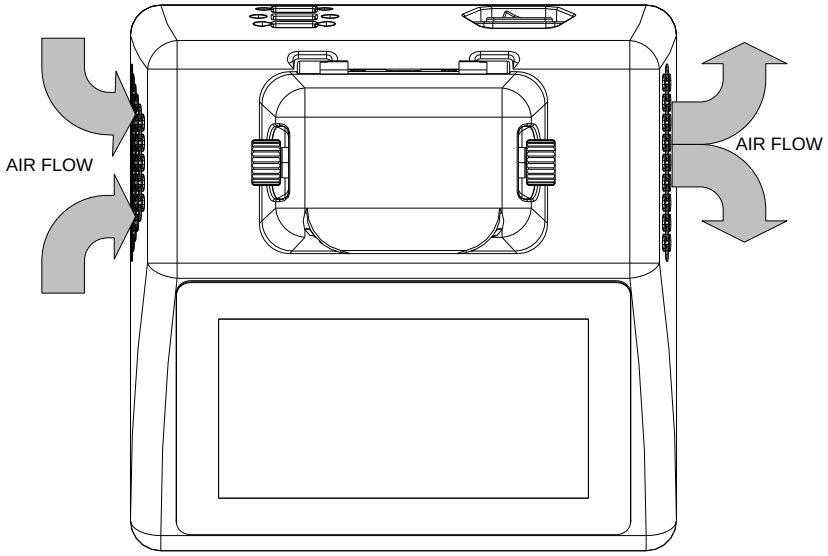


Figure 6 - Air flow at the sides of the device

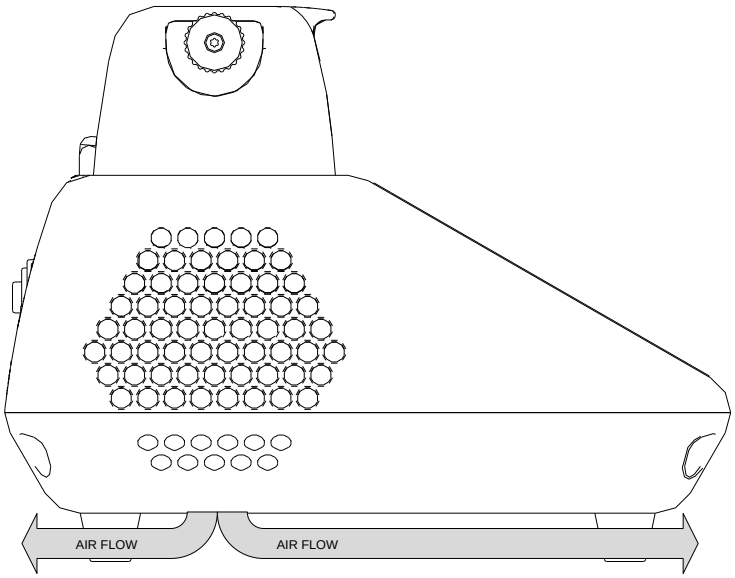


Figure 7 - Air flow at the bottom of the device

Consumables

Here is the list of consumables needed with the device:

- 4-Well PC tube strip prefilled with reagents
- Gold Nanorods solution ready to use
- Nasopharyngeal swabs & universal media

Setup

Unpacking the P-RT-PCR-P4

Carefully lift the device from the shipping box. Place it on a flat surface and remove the packaging foam.

NOTE: Please keep box and packaging in case of return or shipping to a different location.

Remove all the accessories from the box.

Check to ensure that all components are present and intact:

- Plasmonic P-RT-PCR portable unit
- Three (3) prong power cord
- Manual
- Ethernet Cable
- Temperature sensor calibration kit

P-RT-PCR-P4 Placement

For proper ventilation, place the unit in an unimpeded space of at least 5cm (2”) at the front and the back of the unit.

Make sure the unit has a minimum of 8cm (3”) of space above the unit for the easy and safely manipulation of the lid.

Provide easy access to the back of the unit for the power switch.

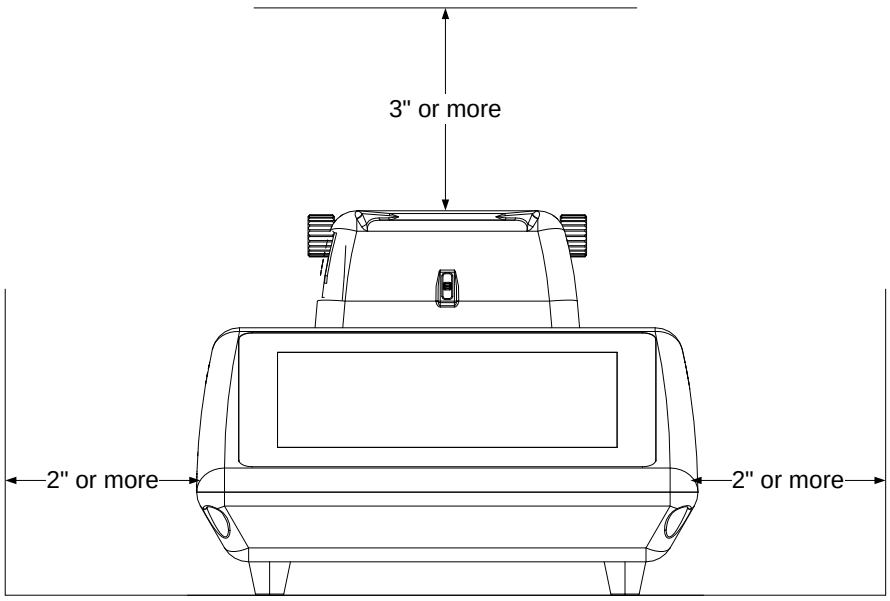


Figure 8 - Minimum space required around the device

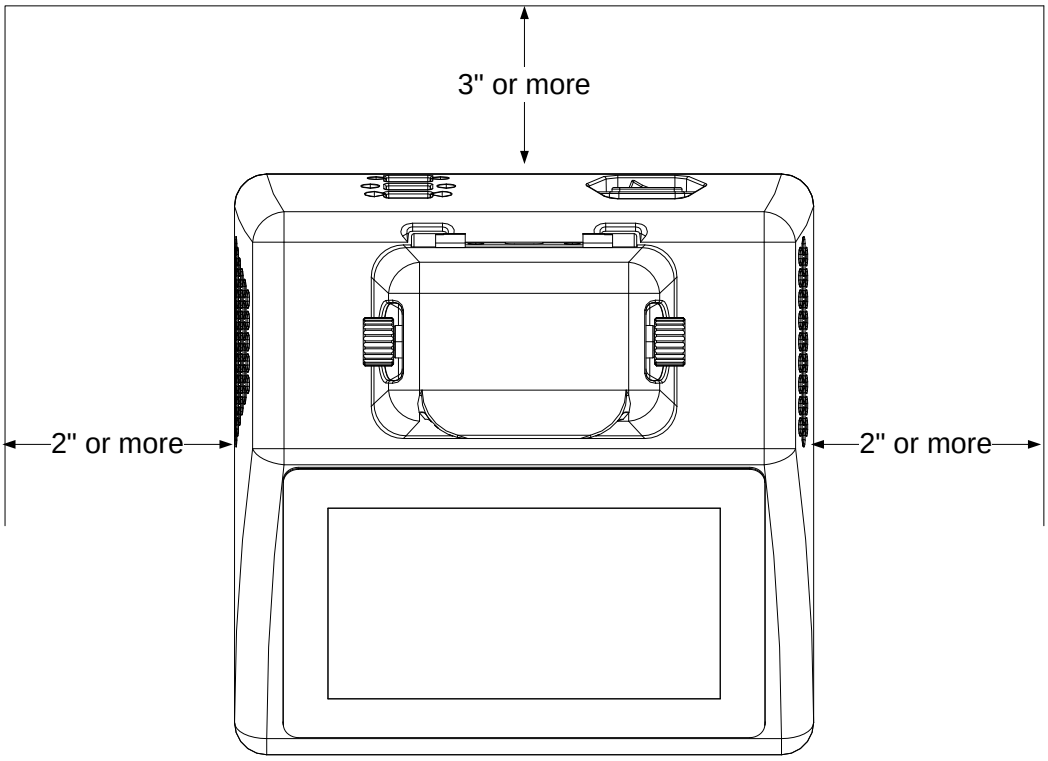


Figure 9 - Minimum space required around the device

Initial Setup

Connect the three (3) prong power cord to power inlet at the back of the unit, and then to the wall power outlet.

Turn-on the power switch of the back of the unit. The unit will power-up and perform a self test. The self test should take approximately 1 minute. Once done, the Welcome screen should display on the touch screen.

The unit arrives calibrated and ready to use directly from the factory. It has been calibrated for the Progene PCR tubes. No calibration is required before usage if the same tubes are used.

You are now ready to use the PCR unit.

Workflow Procedure

PCR Protocol

Here is some precaution before starting a new test:

- Wear a clean lab coat, clean gloves, eye and face protection when preparing samples for PCR amplification.
- Ensure there are separate areas for PCR setup and PCR amplification. Avoid bringing amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Avoid splash-back of the PCR samples.
- Use aerosol resistant pipette tips and change tips after each use.
- Ensure that reaction and reagent components are always capped when not in use.
- Clean lab benches and equipment periodically.

Control Procedure

It is a good practice to include a positive control and a negative control at the beginning of every testing batch. The controls could be used on a test batch basis.

The positive control contains the following:

- Luna Probe One-Step RT-qPCR 4X Mix with UDG 5 µl
- SARS-CoV-2 Primer/Probe Mix (N1/N2/RP) (10X) 2 µl
- SARS-CoV-2 Positive Control (N gene) 2 µl
- GNR 1 µl
- Nuclease-free Water to complete 20 µl 10 µl
- Reaction (0.2 ml) tubes 4 tubes

The positive control will confirm the PCR test process.

The negative control contains the following:

- Luna Probe One-Step RT-qPCR 4X Mix with UDG 5 µl
- SARS-CoV-2 Primer/Probe Mix (N1/N2/RP) (10X) 2 µl
- GNR 1 µl
- Nuclease-free Water to complete 20 µl 12 µl
- Reaction (0.2 ml) tubes 4 tubes

The negative control will confirm that there is no contamination of the SARS COV-2 reagents.

PCR procedure

RT-PCR technique is the gold standard technique to test for infectious diseases such as the screening of COVID-19. Here is the procedure to follow:

1. Prepare the following components:
   - Luna Probe One-Step RT-qPCR 4X Mix with UDG 5 µl
   - SARS-CoV-2 Primer/Probe Mix (N1/N2/RP) (10X) 2 µl
   - GNRs 1 µl
   - Reaction (0.2 ml) tubes up to 4 tubes
2. Use a swab to take a sample of the upper respiratory specimen from the patient. The virus usually gathers at the back of the nose or throat. The swab should go deep enough to get adequate amount of potential virus.
3. Put the swab in a process tube (1 ml tube or bigger) containing a hot Nuclease free water. Stir for at least 10 seconds.
4. Pipette out 12 µl of the content of the process tube and put it in the reaction tube.
5. The viral RNA is converted to cDNA using an enzyme, the reverse transcriptase. During this process, the content of the vial is heated to approx. 48 degrees for 5 minutes. Once the reverse transcription process is complete, the amplification process starts.
6. Amplification process uses reagents containing primers, fluorescent labels, nucleotides, enzymes, and gold nanoparticles. These reagents mixed with the DNA specimen will duplicate DNA as follows:
   - At denaturing stage (around 85 degrees), the DNA will separate into single strands.
   - At around 60 degrees, at annealing step the primers initiate the replication process.
   - At 72 degrees, at elongation (or extension) step the enzymes complete the copies of the strands.
   - These steps are repeated up to 40 cycles to get a few million copies of the viral genetic material.
7. During the annealing cycle, fluorescence level is measured to detect the amount of viral genetic material.
8. Three screening tests are available: Rapid Covid, Fast Covid and Ultrafast Covid.
   - The Rapid covid test is a 25-minute (or less) test that should rule out the presence of the virus. The sensitivity is 100 copies.
   - The Fast Covid test is a 15-minute (or less) test to detect 1,000 copies or more.
   - The Ultrafast Covid test takes less than 10 minutes and has a limit of detection of 100,000 copies.

Sample Preparation

Extracted DNA or RNA is the starting material for sample preparation. The quality of the extracted DNA or RNA has a profound impact on the performance of the entire test system. It must be ensured that the system used for nucleic acid extraction is compatible with real-time Plasmonic PCR technology.

Covid samples

For each of the samples, prepare the following 20 µL solution:

- RNA[1] 5.6 µL
- Luna Probe One-Step RT-qPCR 4X Mix with UDG[2] 10.0 µL
- 10 µM Forward Primer[3] 0.8 µL
- 10 µM Reverse Primer[3] 0.8 µL
- 2.5nM Gold Nanorods[4] 1.0 µL

The unit will provide testing and results for up to four tests at one time.

[1] RNA of extracted patient sample with ddH_2O.
[2] Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB Cat# M3019).
To the 4X reaction buffer, we add SYTO-16 (a fluorescence dye, similar to SYBR Green) at a final concentration of 8uM.
[3] Forward Primer: 5’ AGATCACATTGGCACCCGCAA 3’;
Reverse Primer: 5’ CCGCCATTGCCAGCCATTCT 3’.
[4] Gold Nanorods (Nanopartz Cat# C12-10-808-PEG-DIH-50).

Chlamydia Samples

For each of the samples, prepare the following 20 µL solution:

- DNA[1] 11.3 µL
- 5X Reaction buffer[2] 4.0 µL
- Klentaq DNA polymerase[2] 1.0 µL
- 2.5nM Gold Nanorods[3] 1.0 µL
- 10 µM Forward Primer[4] 0.6 µL
- 10 µM Reverse Primer[4] 0.6 µL
- dNTPs (10 mM) 0.5 µL

[1] DNA of extracted patient sample with ddH_2O.
[2] Hemo Klen Taq – supplied with 5X Hemo Klen Taq Reaction buffer (NEB Cat# M0322).
[3] Gold Nanorods (Nanopartz Cat# C12-10-808-PEG-DIH-50).
[4] Forward Primer: 5’-TCCGGAGCGAGTTACGAAGA-3’;
[4] Reverse Primer: 5'-AATCAATGCCCGGGATTGGT-3'.

E-Coli samples

For each of the samples, prepare the following 20 µL solution:

- RNA[1] 5.6 µL
- 2X Reaction buffer[2] 10.0 µL
- Protoscript II RT[3] 0.8 µL
- Taq DNA polymerase[4] 0.8 µL
- RNase Inhibitor[5] 0.2 µL
- 10 µM Forward Primer[6] 0.8 µL
- 10 µM Reverse Primer[6] 0.8 µL
- 2.5nM Gold Nanorods[7] 1.0 µL

[1] RNA of extracted sample.
[2] 2X reaction buffer from OneTaq One Step RT-PCR kit (NEB Cat# E5315S).
To the 2X reaction buffer, we add SYTO-16 (Thermo Fisher Cat# S7578) at a final concentration of 8uM.
And Total E.coli RNA (Thermo Fisher Cat# AM7940) at a final amount of 10ng.
[3] Protoscript II Reverse Transcriptase (NEB Cat# M0368).
[4] Taq DNA polymerase (NEB Cat# M0267).
[5] RNase Inhibitor Murine (NEB Cat# M0314).
[6] Forward Primer: 5' TCTGTCTTCTGGCTTGCGTATTAAC 3';
Reverse Primer: 5' CACACGCTGTAAGTTGTTGTTGATT 3'
[7] Gold Nanorods (Nanopartz Cat# C12-10-808-PEG-DIH-50).

Good practices

For specimen collection and transport, personnel shall personal protective equipment for providing a barrier to help prevent potential exposure to infectious disease. Personal protective equipment includes medical gloves, isolation gowns, surgical masks, face shields, and goggles for eye protection.

Collected specimens should be in containers tightly closed, placed, and sealed in a biohazard bag identified with a label.

Please contact your local health authorities for rules and regulations.

Testing Procedure

Once the samples have been prepared and are ready for testing, follow the following steps:

- Insert the test tube strip onto the test tube holder
- Cover the test tube with cap
- If necessary, adjust the lid height with the adjustable knobs
- Close the lid

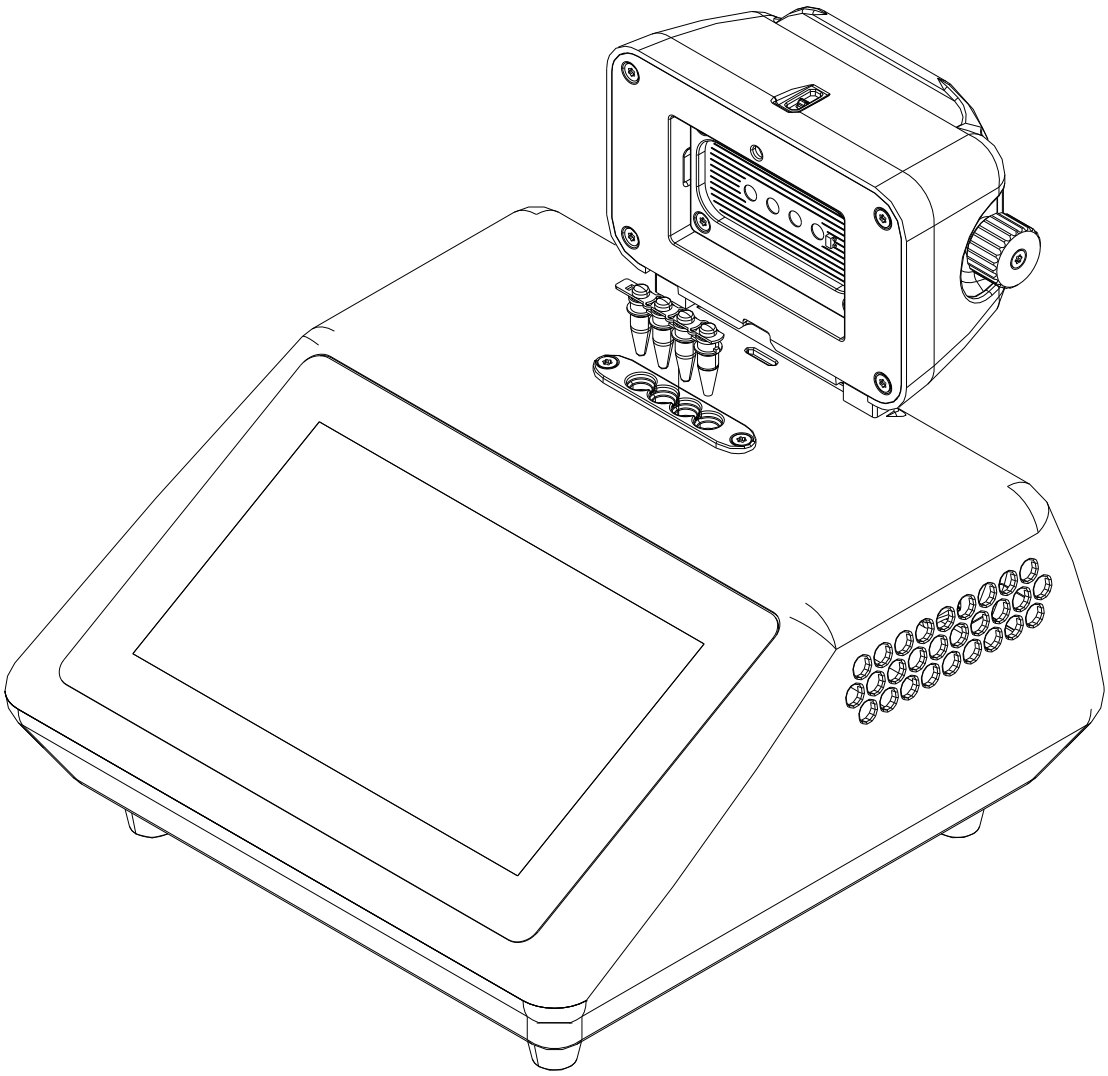


Figure 10 – Inserting the test tubes in the device

Test results are saved locally and can be retrieved using the LAN connection.

Software Procedure

Follow the following procedure on the touch screen to perform the proper test for the patient samples.

Normal Operations

The device is already loaded with the protocols for SARS-Cov2 and Chlamydia testing assays.

Login screen

- After powering the device, a full self test is performed. If an error occurs, it will be displayed in red.

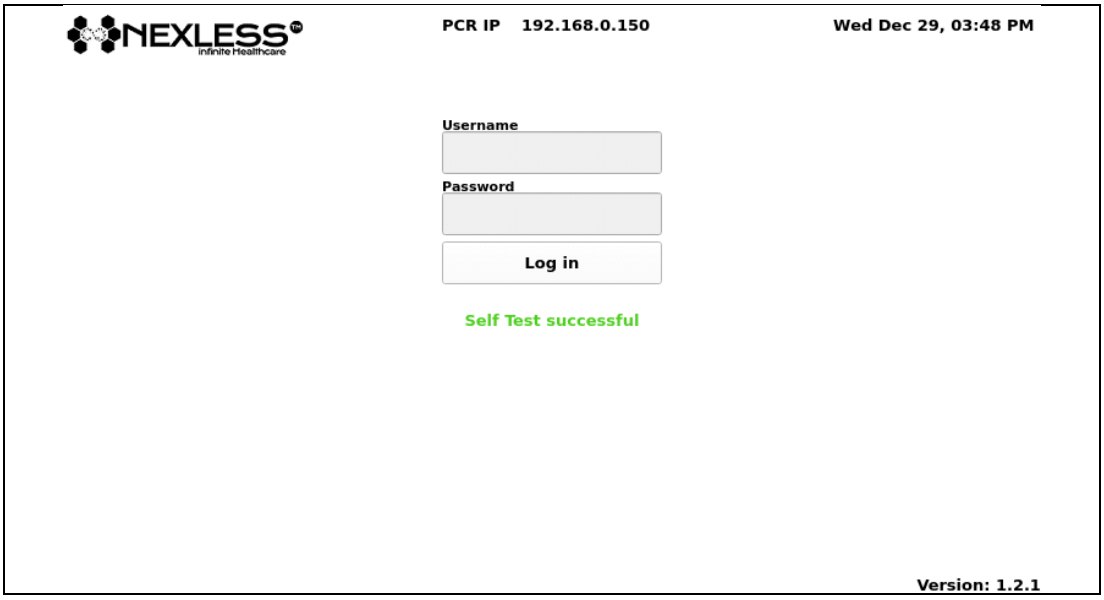


Figure 11 – Self test result displayed

- After the self test, you will be directed to the login screen. By default, the user ID is *admin,* and the password is *admin.*

Figure 12 – Login screen

PCR Protocol page

- After login, you will be directed to the PCR Protocols screen. The device is preloaded with two protocols: the CTC (Chlamydia) and the COVID-19) protocols. Up to 10 protocols are displayed per page. You can navigate through the PCR protocols pages by clicking on “Next Page” or “Previous Page” buttons. When a protocol is chosen, it is indicated at the lower left corner of the screen. After a protocol is chosen, you can click on the “NEXT” button to go to the test ID screen. Please note that the lower portion indicates the result of the latest simple self test. When clicked, the “Settings” icon ⚙ at the top right corner will direct you to the settings screen. For convenience, the IP address of the PCR is displayed.
  The Log Manager page lists the history of the past PCR tests. To access the Log Manager, click on the icon. Please refer to the Log Manager section.

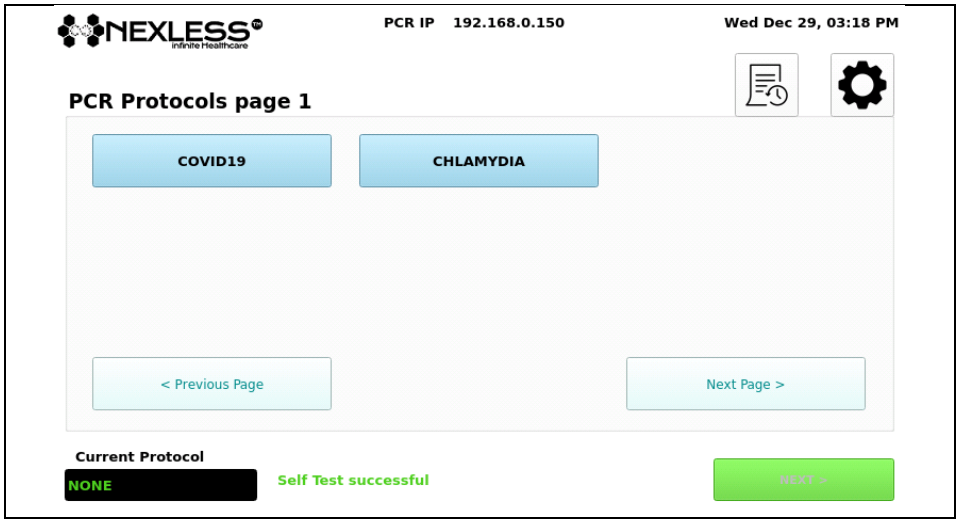


Figure 13 – PCR Protocols screen

- At the test tube identification screen, you can enter the tests identification. A name, test ID or patient ID can be used. A bar code scanner can also be used to easily enter the identification.

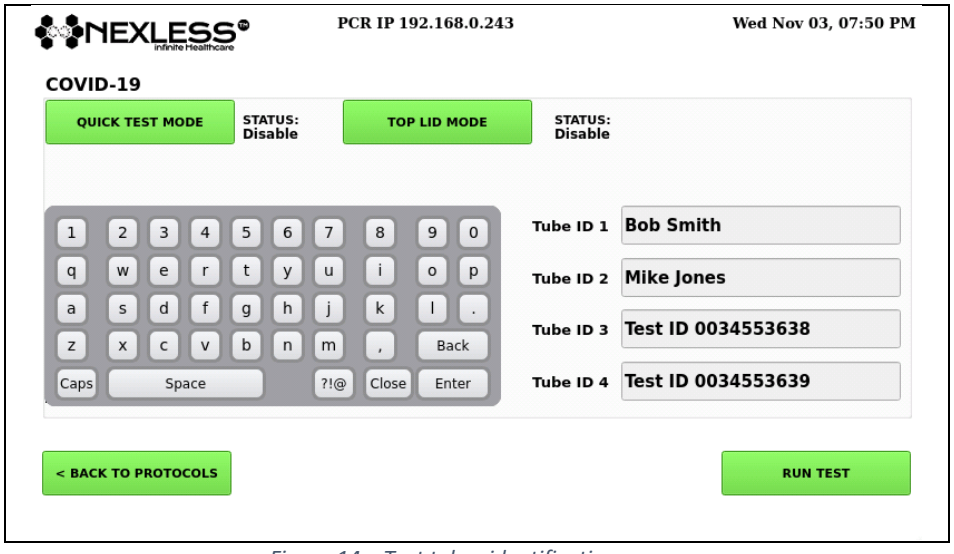


Figure 14 – Test tubes identification screen

- Heater Lid: The PCR-P4 is equipped with a heater lid to keep the top of the test tube hot enough and avoid evaporation. Another way to avoid evaporation is to add 20uL of mineral oil on top of the sample. The heater lid will take about 5 minutes before starting the PCR test. From the test menu, it can be enabled. To change the Top lid mode, just press on the “TOP LID MODE” to toggle the status:

- Early detection: The PCR-P4 can detect a positive PCR reaction before the end of the PCR thermocycles as soon as exponential amplicons amplification is detected. The Early Detection option can be enabled from the Test menu. If the early detection is enabled, the test will stop two cycles after the threshold cycle, reducing the PCR test time. To enable the Quick test mode, just press the “QUICK TEST MODE” button to toggle the status:

- Click “RUN TEST” to start the test. If the Top lid is enabled, the temperature of the top lid will be displayed. The PCR step, the temperature values, the time elapsed, the quantization values, the cycle number, and the progress bar are displayed. The test can be cancelled at any time. Do not open the top lid as it will affect the PCR process.

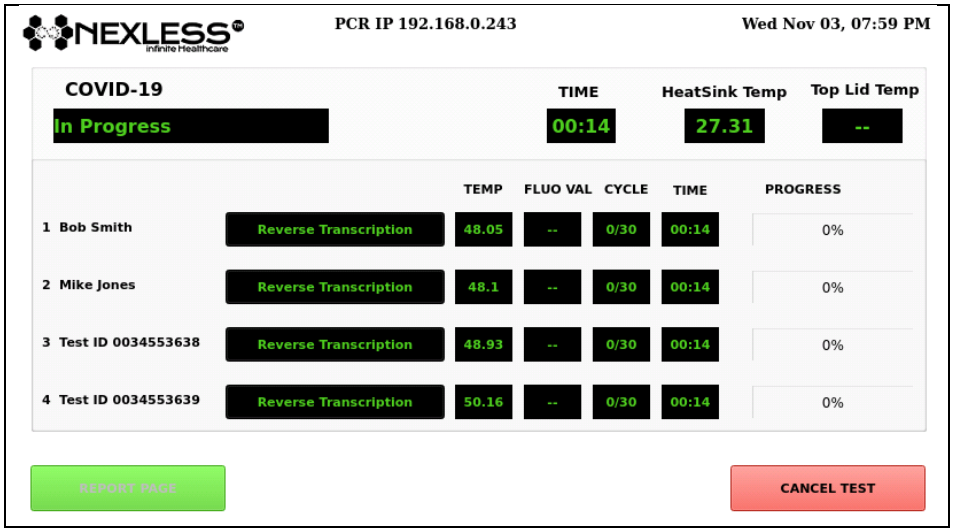


Figure 15 – “Test started” screen

- Once complete, you will have access to the report page. Results are either Positive, Negative, or not applicable. If positive, the threshold cycle (Ct) will be displayed.

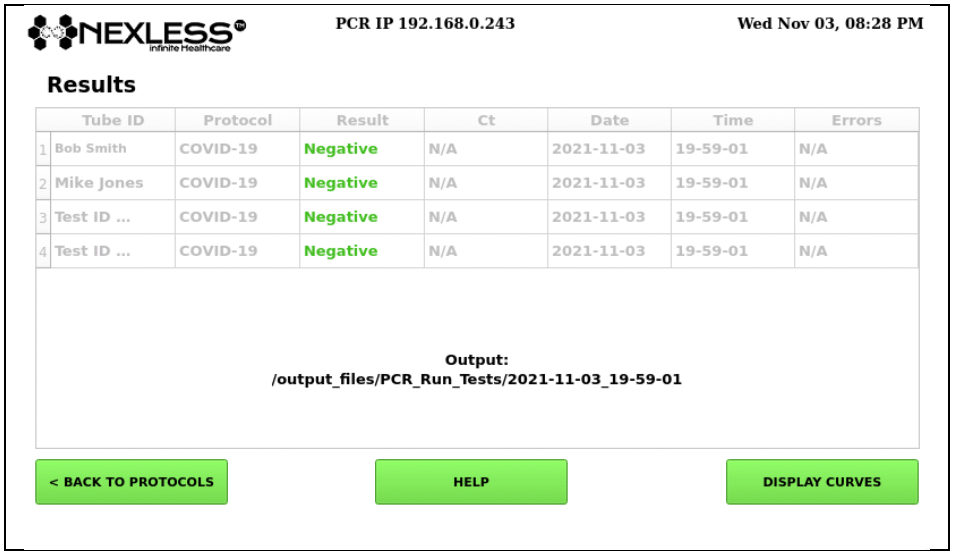


Figure 16 – Results screen

- Pressing “Display curves” will list the temperature cycles and fluorescence curves available. Choose the curve to be displayed. Press “CLOSE” when finished.

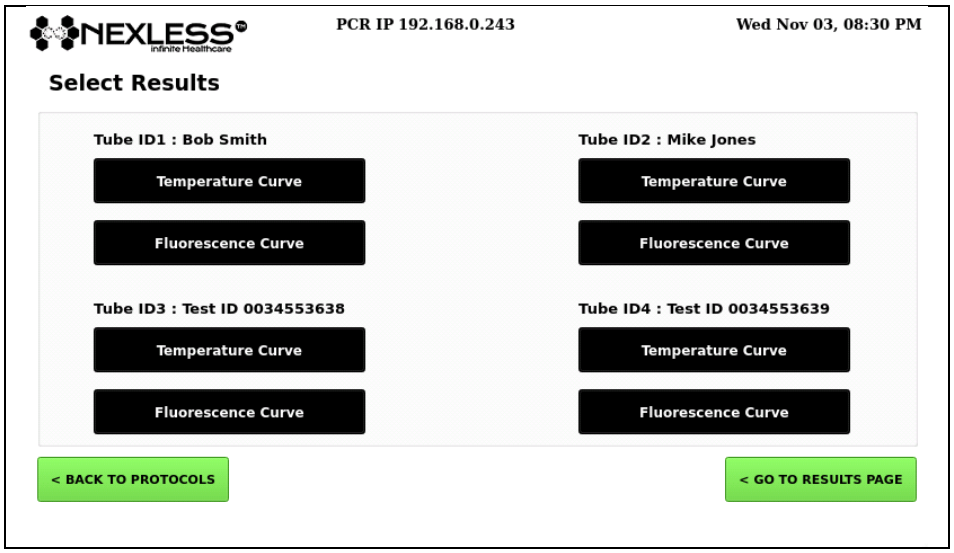


Figure 17 – Example of a temperature cycles curve displayed on screen

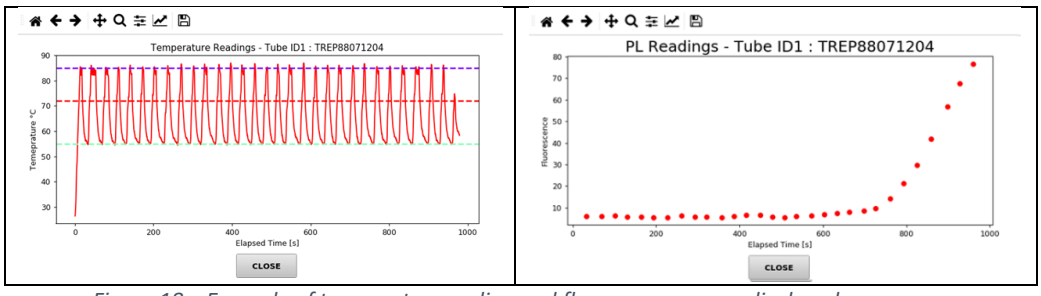


Figure 18 – Example of temperature cycling and fluorecence curves displayed on screen

- The report is available at the location indicated on the Results screen. Pressing “HELP” will give you the steps to setup your computer to access the reports. Reports are not automatically deleted; it is a good practice to delete the data once it is downloaded on your computer.

  Important note: When doing a software update, all test results data will be erased.

  The test report is available in XML format and can be viewed and printed from your favorite browser. Below is an example of a 3-page report.

  The first page of the report will show the information on the assay being performed (see Figure below).

Nexless P-IV Kimera

LAB Segment Report Direct amplification CHLAMYDIA

Assay Name :	CHLAMYDIA	Run Name :	2021-09-03_13-51-25
Tested By :	Not activated on this version	Report By :	Not activated on this version
Lot Number :	Not activated on this version	Lot Expiration :	Not activated on this version
Instrument :	RTPCR P-IV	Software :	1.1.4A+
Serial Id :	RTPCR P-IVV4202107LAB00031	Test Date :	13H51 03-September-2021

Test Resume:

Cycling Parameter	wedge 1	wedge 2	wedge 3	wedge 4
Assay Name	CHLAMYDIA	CHLAMYDIA	CHLAMYDIA	CHLAMYDIA
RTStep	Not used	Not used	Not used	Not used
Cycle Count	30/30	30/30	30/30	30/30
Actual Run Time	16:51 min	16:51 min	16:51 min	16:27 min
Results	POSITIVE	POSITIVE	POSITIVE	NEGATIVE

PCR Protocol: CTC

Initial Cycles	Times (sec)	Temperature (°C)	Ramp Rate (°C/sec)	
Hot Start	Not used	Not used	Not used	
RT Steps	Not used	Not used	Not used	
Hold 2	Not used	Not used	Not used	
Hold 2	Not used	Not used	Not used	
Main Cycle	Times (sec)	Temperature (°C)	Ramp Rate (°C/sec)	
Denaturing	1	85	5.0	
Annealing	5	55	5.0	
Elongation	1	72	5.0	
Sync	N/A	N/A	N/A	
Extend 1	Not used	Not used	Not used	
Extend 2	Not used	Not used	Not used	
Final Cycles	Times (sec)	Temperature (°C)	Ramp Rate (°C/sec)	
Final 1	Not used	Not used	Not used	
Final 2	Not used	Not used	Not used	

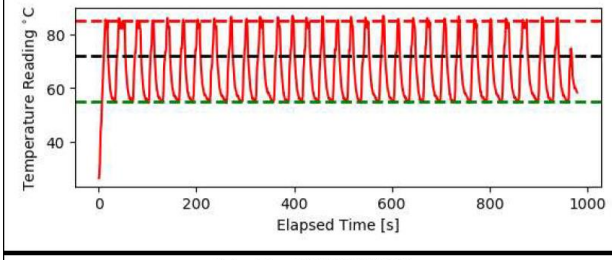
Figure 19 – Example of the XML report file, page 1 of 3

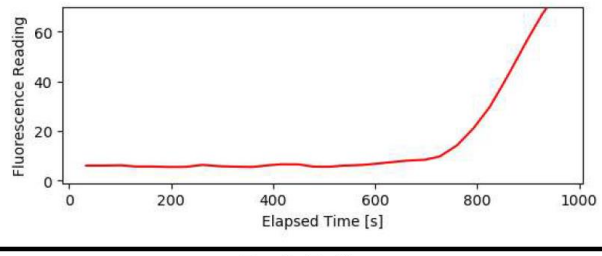
The second page of the report will show the thermocycling and fluorescence curves for each of the tubes being tested.

The third page of the report is a table to enter the list of reagents used in the PCR.

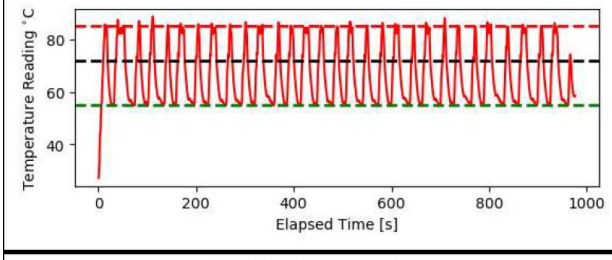
Nexless P-IV Kimera

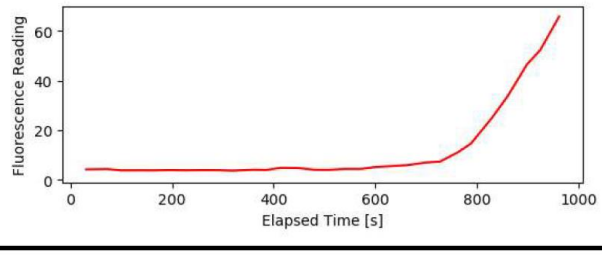
LAB Segment Report Direct amplification CHLAMYDIA



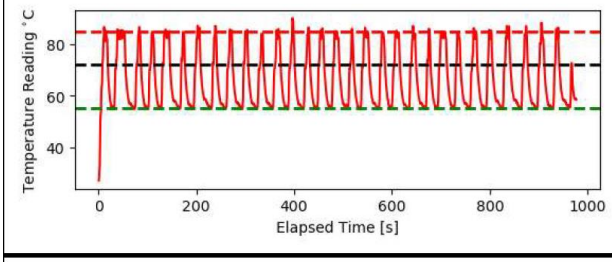


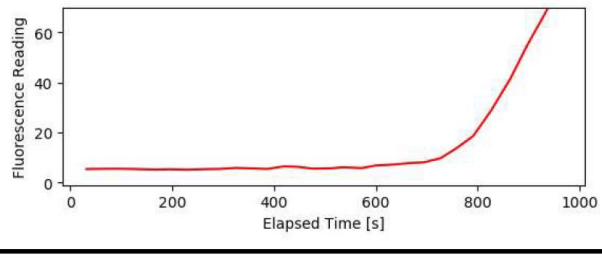
Tube ID = TREP88071204	Result = Positive
Actual Run Time = 16:51 min	CT Value = 24
Wedge 1 - CHLAMYDIA	**Cycles 30/30**



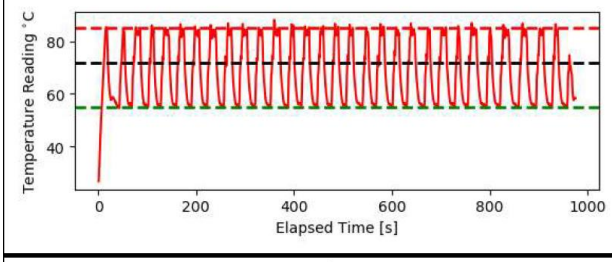


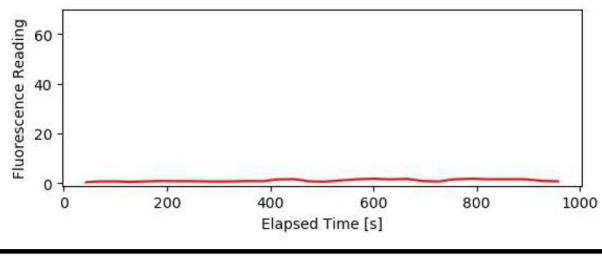
Tube ID = ROYC78122501	Result = Positive
Actual Run Time = 16:51 min	CT Value = 24
Wedge 2 - CHLAMYDIA	**Cycles 30/30**





Tube ID = GIRP96012112	Result = Positive
Actual Run Time = 16:51 min	CT Value = 24
Wedge 3 - CHLAMYDIA	**Cycles 30/30**





Tube ID = LAFG68040623	Result = Negative
Actual Run Time = 16:27 min	CT Value = N/A
Wedge 4 - CHLAMYDIA	**Cycles 30/30**

Figure 20 – Example of the XML report file, page 2 of 3

Nexless P-IV Kimera

LAB Segment Report Direct amplification CHLAMYDIA

RT-PCR reaction of the One Taq One-Step RT-PCR-kit
Axygen® 0.2mL Polypylene PCR Tube Strips and Domed Cap Strips

Components	Volume	Notes
2X Reaction buffer + SYTO-16	10uL	The 2X reaction buffer we modified: 496uL buffer + 4uL 1mM SYTO-16, with a final concentration of 8uM of SYTO-16
Forward	0.8ul(300nM)	
Reverse	0.8ul(300nM)	
Tag Polymerase (5U/uL)	0.8ul(4U)	Neb (M0627) - 5,000U/mL
Protoscript II(200U/uL)	0.8ul(16U)	Neb (M0368) - 200,000U/mL
RNase Inhibitor(8U/uL)	0.2ul(8U)	Neb Murine (M0314) - 40,000U/mL
GNR (50 OD)	1.0uL (2.5nM)	
Template	4uL	swab max volume
d2H20	(delta)	
Total Volume	**20uL**	

Figure 21 – Example of the XML report file, page 3 of 3

- If you need help setting up your PC connection to retrieve the report and test data using Samba, click "HELP" on the results page. You can now navigate through the steps:

Step1 & 2: If not already done, activate Samba on your PC.

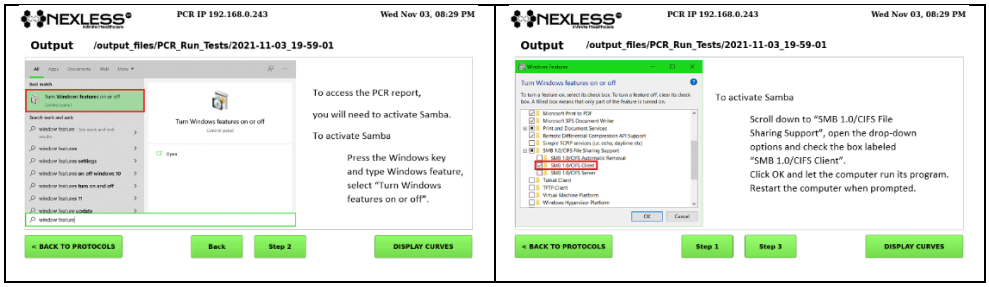


Figure 22 – Activate Samba software on Windows, Step 1 and 2

Step 3 & 4: Open Windows Explorer and map the network drive of the device.

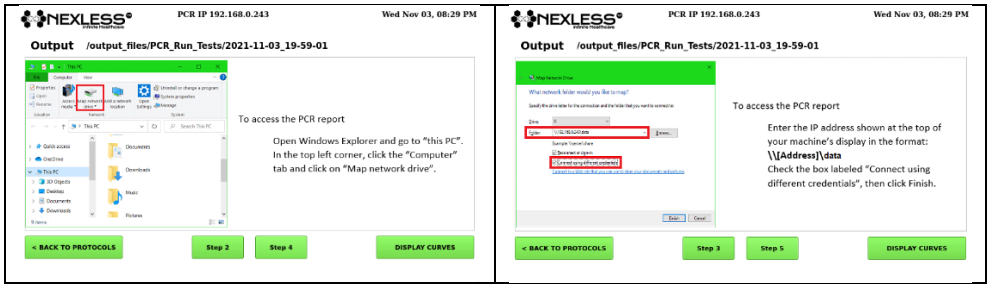


Figure 23 – Activate Samba software on Windows, Step 3 and 4

Step 5 & 6: Enter your credentials and view the list of reports.

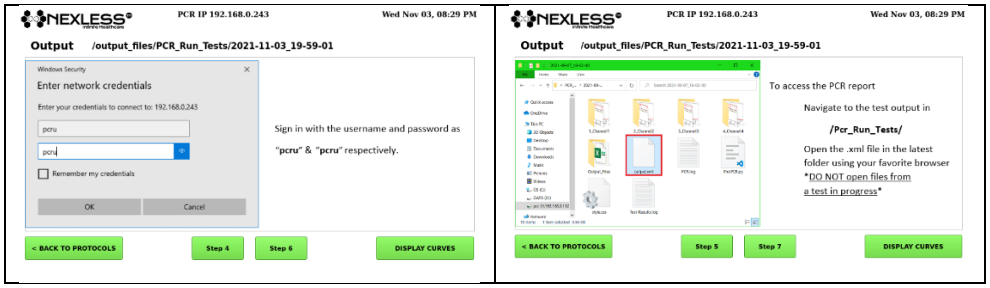


Figure 24 – Activate Samba software on Windows, Step 5 and 6

Step 7: Reports are listed by date and time of the test.

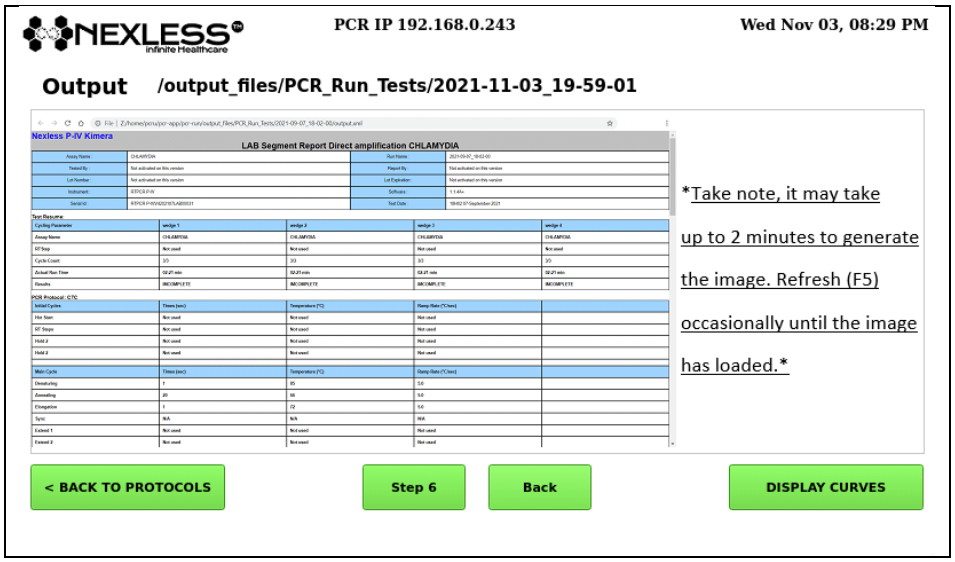


Figure 25 – Activate Samba software on Windows, Step 7

Settings Menu

At any time, you can access the "Settings" menu by clicking on the "Settings" icon ⚙ at the top right corner. The settings menu helps you with admin operations, PCR protocol management, time zone settings, Screen brightness settings, software update, temperature calibration, and more.

Admin Operations

The PCR-P4 device supports user login to prevent unauthorized use of the device. To login to the device for the first time, you will use the following User ID and password:

- User ID: admin
- Password: admin

Note: This feature will be available on future software version.	Once logged in the system, you can create new user IDs and passwords by entering the Account Manager page. Once you create the first User ID / Password combination, the initial admin user ID will be deleted. Be careful to use a User ID / Password combination that you will remember.

Creating a PCR protocol

The PCR device comes preloaded with two protocols: the CTC (or Chlamydia), and Covid-19 (or SARS-Cov2). However, more protocols can be added.

Before creating a new test protocol, you need to identify the parameters of the new protocol to create. The parameters needed are:

- Synchronisation:
  - The **Synchronisation step** is generally added to synchronise all the channels and speed up the thermocycles. Default is ON or enabled.
  - **Synchronisation temperature** should be set to 2°C below the denaturation temperature setting.
  - **Synchronisation temperature tolerance** is set by default to 1°C.

- Reverse Transcription (RT):
  - The **Reverse Transcription** should be set to ON or OFF. If set to ON, the PCR will perform a reverse transcription step prior to starting thermocycling.
  - **Reverse Transcription temperature** can be set to a value between 45°C and 55°C. Default is 48°C.
  - **Reverse Transcription duration** is set by default to 300 seconds.
  - **Temperature tolerance for Reverse Transcription** is set by default to 1°C.
- Denaturation:
  - The **Denaturation temperature** default value is 85°C.
  - **Denaturation step duration** default value is 1 second.
  - **Denaturation temperature tolerance** default value is 1°C.
- Annealing:
  - The **Annealing temperature** default value is 60°C.
  - **Annealing step duration** default value is 5 second.
  - **Annealing temperature tolerance** default value is 1°C.
- Elongation:
  - The **Elongation temperature** default value is 72°C.
  - **Elongation step duration** default value is 1 second.
  - **Elongation temperature tolerance** default value is 1°C.
- Number of cycles:
  - The **Number of cycles** default value is 30 cycles.

To create a new protocol, click the “Settings” icon to enter the **Protocol Manager** page, press “Add”.

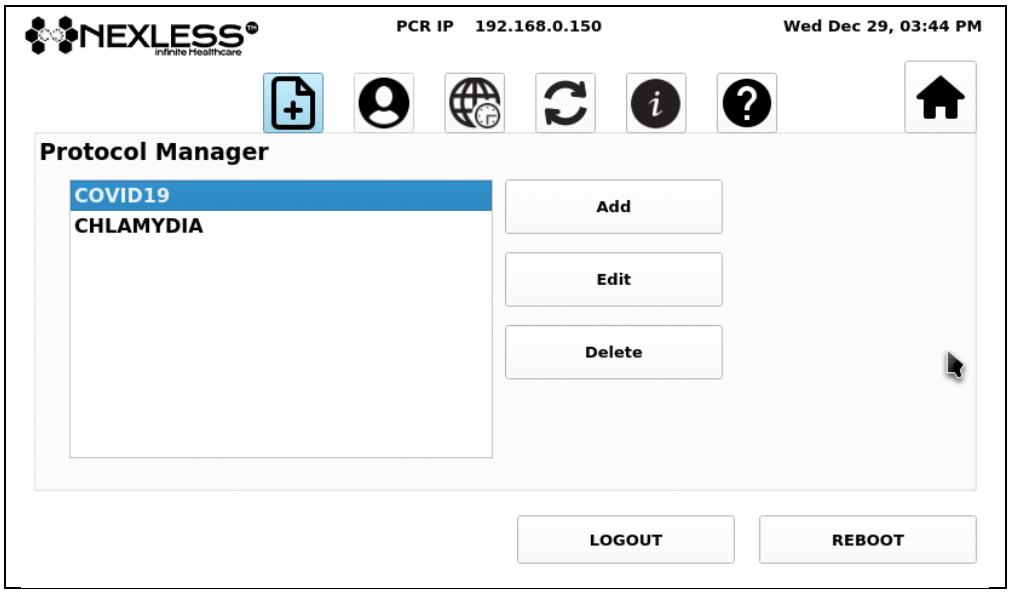


Figure 26 – Protocol Manager page

Enter the name of the new protocol to create and click “Create”.

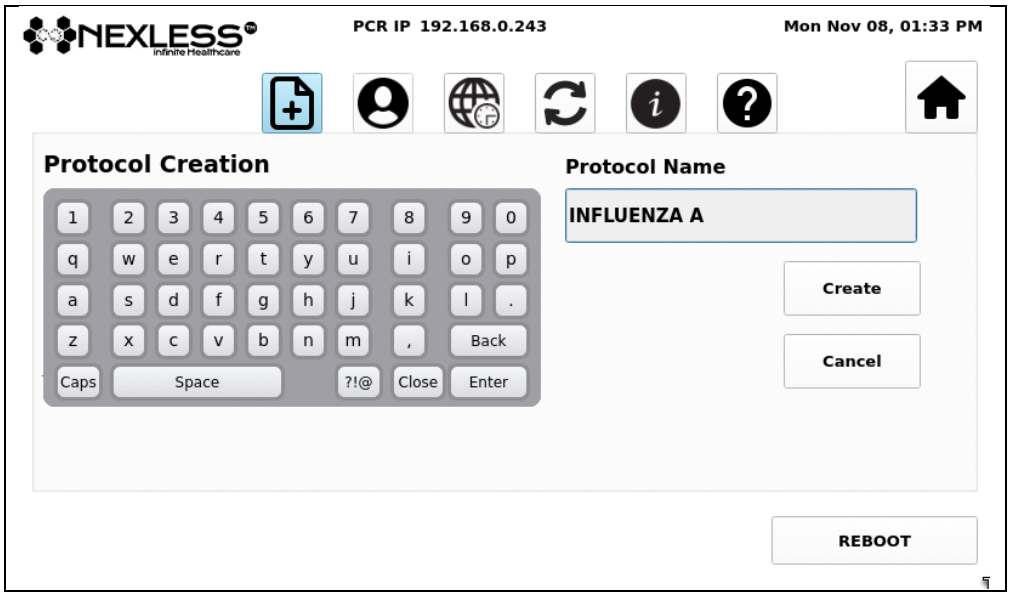


Figure 27 – Creating a new protocol

You can then enter the parameters values listed above and click “Apply” to apply the change and a “Setting Applied” message will be displayed. When all parameters have been changed, click “Save” to save the protocol and a “Protocol Saved” message will be displayed.

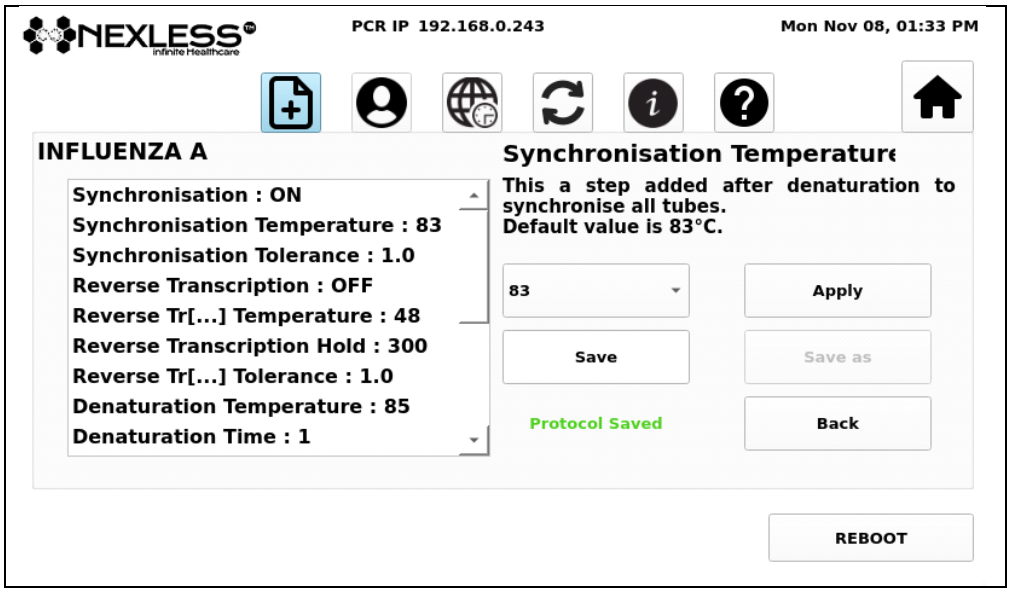


Figure 28 – Creating a new protocol

Once saved, the protocol will be added to the list of available protocols so it could be selected for a test.

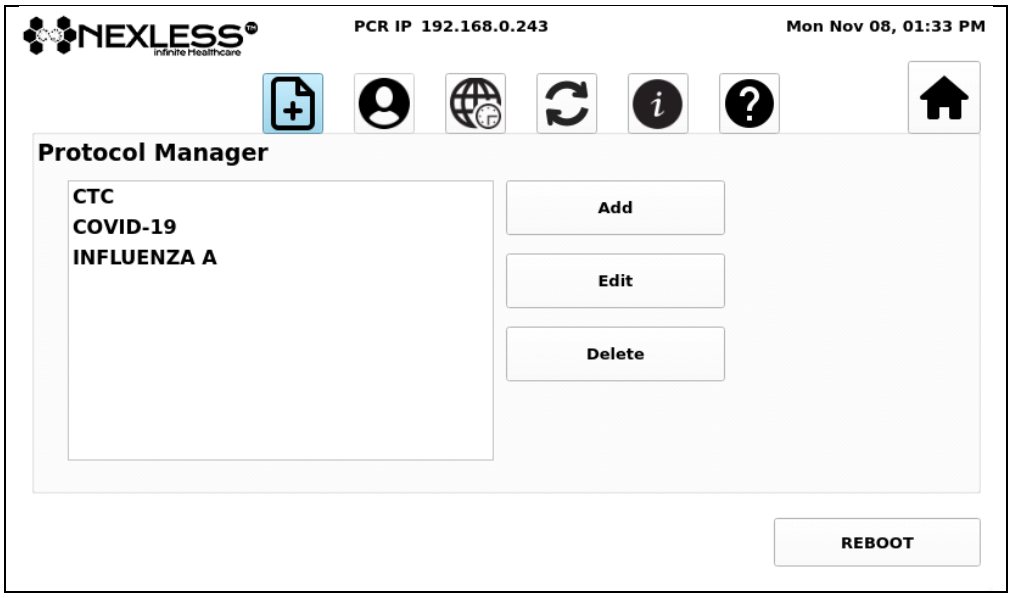


Figure 29 – Creating a new protocol

Click the "Home" button to access the **PCR Protocol** pages.

Log Manager page

All completed PCR tests are kept in memory and can be viewed or downloaded on a USB flash drive. To view the PCR test history, click on the Log Manager icon on the "Home" screen.

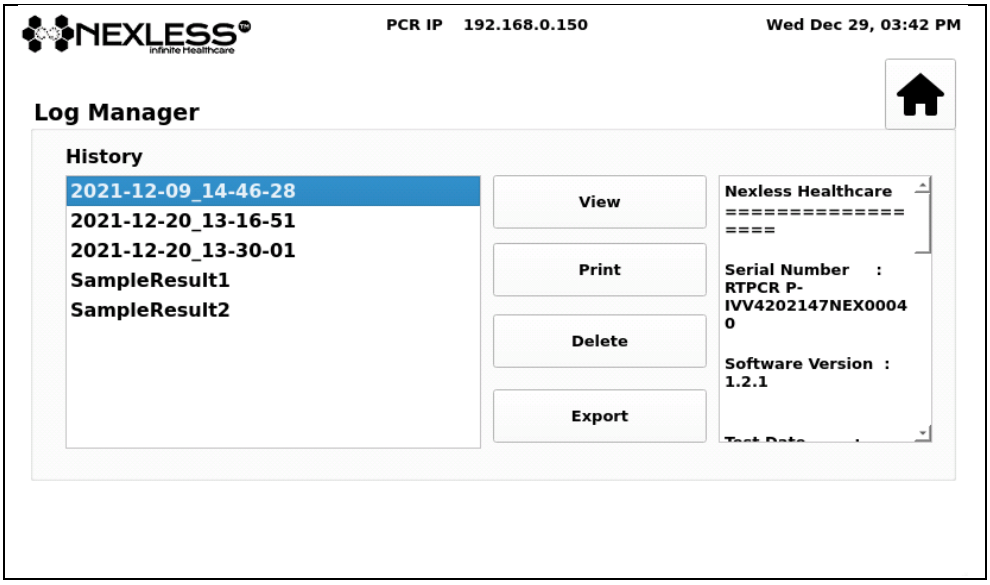
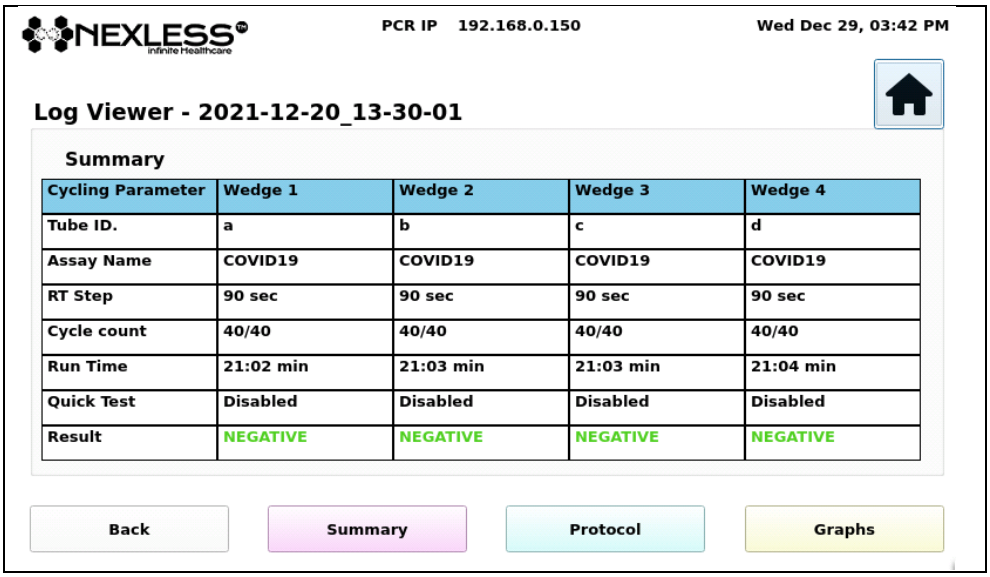


Figure 30 – Log Manager page

To view a test, select the test and click on the “View” button. The system will then display the summary of the test.



Cycling Parameter	Wedge 1	Wedge 2	Wedge 3	Wedge 4
Tube ID.	a	b	c	d
Assay Name	COVID19	COVID19	COVID19	COVID19
RT Step	90 sec	90 sec	90 sec	90 sec
Cycle count	40/40	40/40	40/40	40/40
Run Time	21:02 min	21:03 min	21:03 min	21:04 min
Quick Test	Disabled	Disabled	Disabled	Disabled
Result	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE

Figure 31 – Log Manager Viewer Summary

To see the protocol profile used for this test, click on “Protocol”.

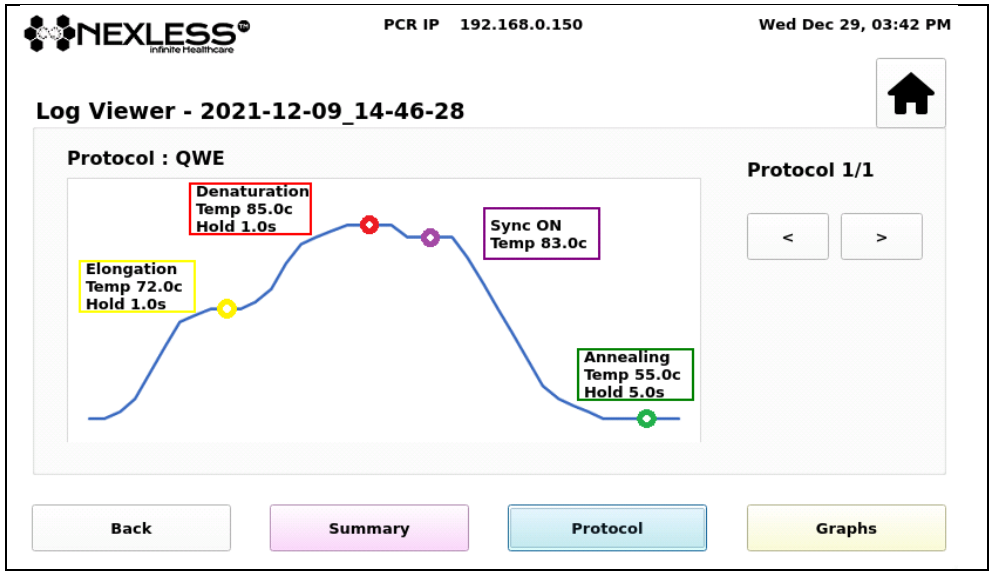


Figure 32 – Log Manager Viewer Protocol profile

The different temperature used on the protocol profile are displayed.

To see the results in a graphical, click on “Graphs”. The two graphs available are the temperature and the fluorescence graphs.

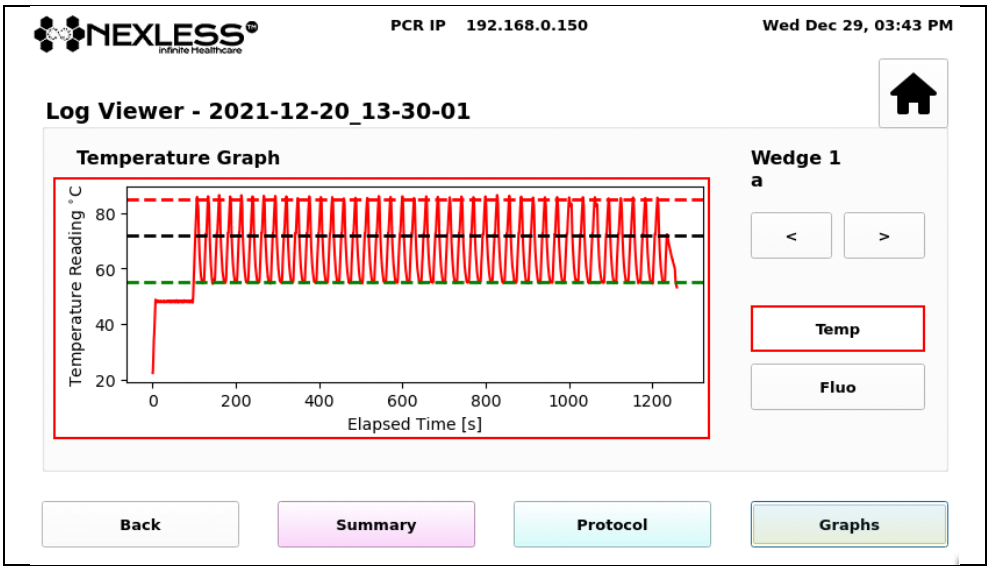


Figure 33 – Log Manager Viewer Temperature Graph

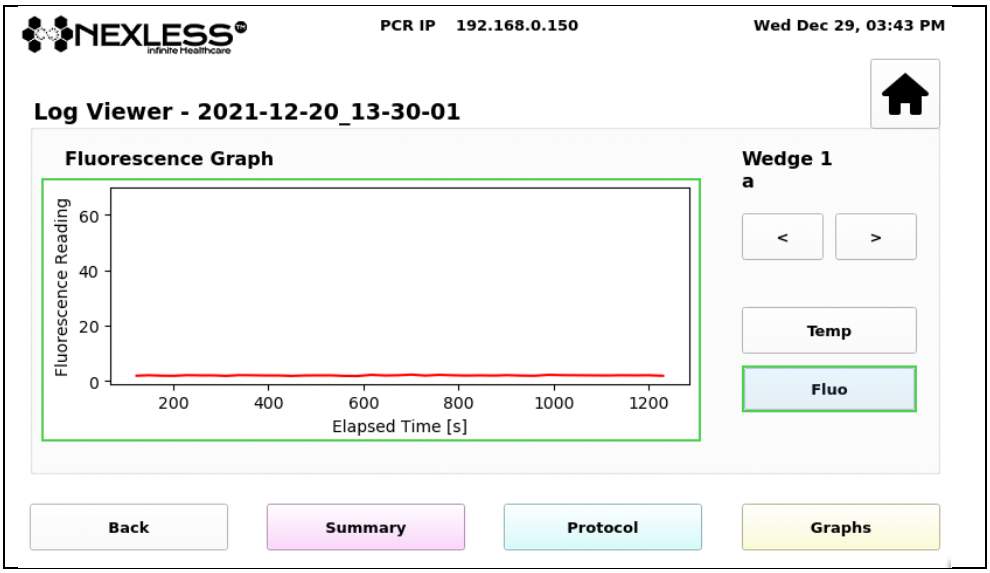


Figure 34 – Log Manager Viewer Fluorescence Graph

Setting the time zone and Screen Brightness

The PCR device’s date and time is updated when an ethernet connection is available. However, to have the date and time that correspond to your geographical area, the time zone should be set accordingly. You can set the time zone by clicking on the time zone icon. Choose the continent, or country and city; then press set to set the time zone. Click save to have the time zone permanently set. The time zone will be adjusted automatically.

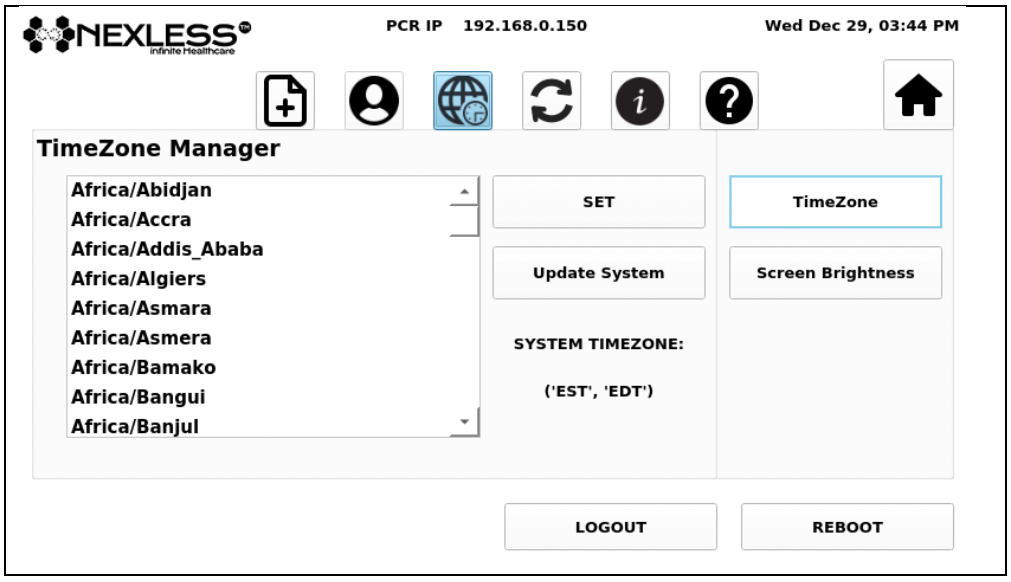


Figure 35 – Time Zone Manager and Screen Brightness Settings page

At the same time, the screen brightness can be set on the same page.

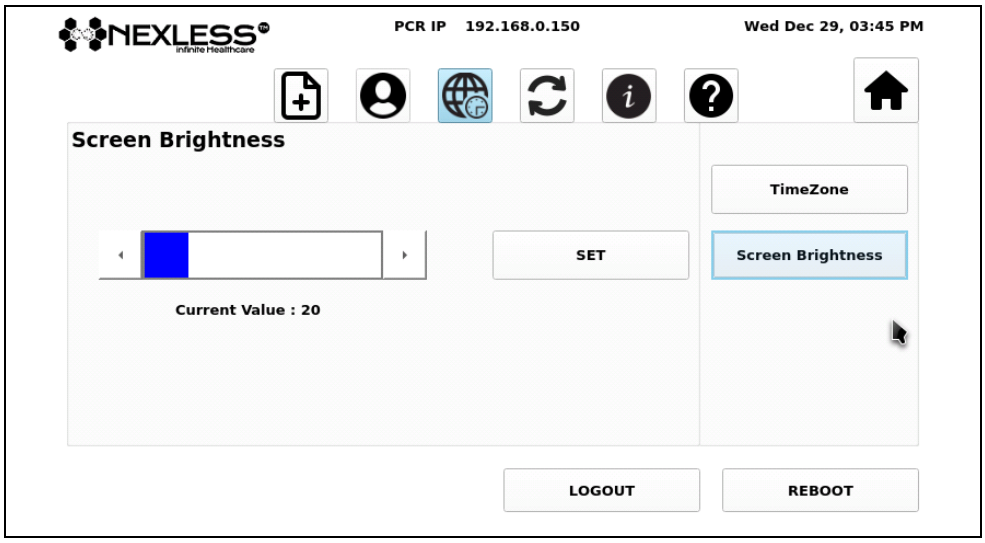


Figure 36 –Screen Brightness Setting page

Software update

The PCR device internal software can be updated with corrections and new features. To update the device software, download the software and copy it on a USB key. Connect the USB key to the USB port at the rear of the device. Click the software update icon to enter the Software Update page. Click "Upload" to upload the software; the device will verify the content of the USB key to make sure the update file is good. Please be patient as this step will take up to 3 to 4 minutes to complete.

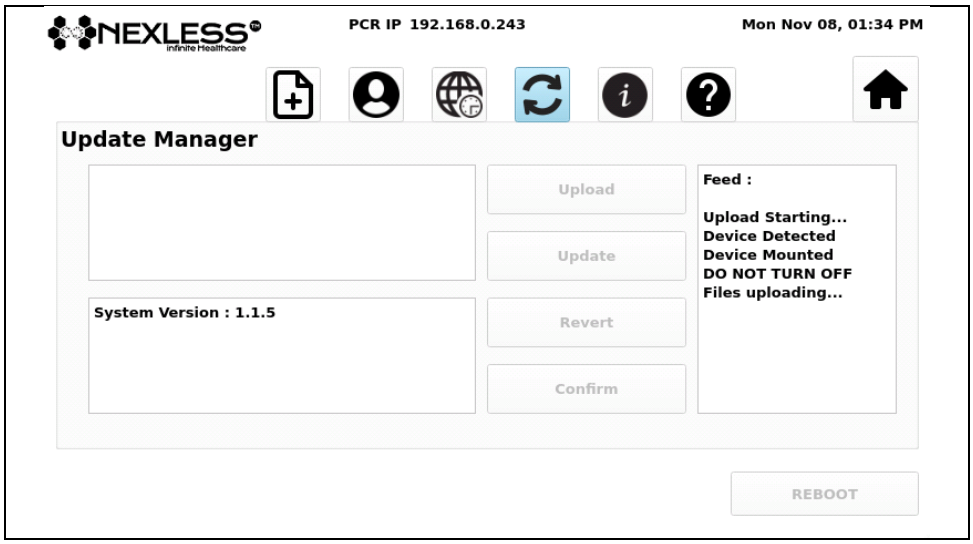


Figure 37 – Software Update Manager page

Once uploading is complete, click “Update”.

Important note: When doing a software update, all previous test results data will be erased.

To complete the update, click “Confirm”. The device needs then to be rebooted for the new software version to take effect. The previous version will be kept on backup within the device.

You can choose at any time to revert to the previous version of the software by clicking “Revert” and then “Confirm”. The device needs then to be rebooted for the new software version to take effect.

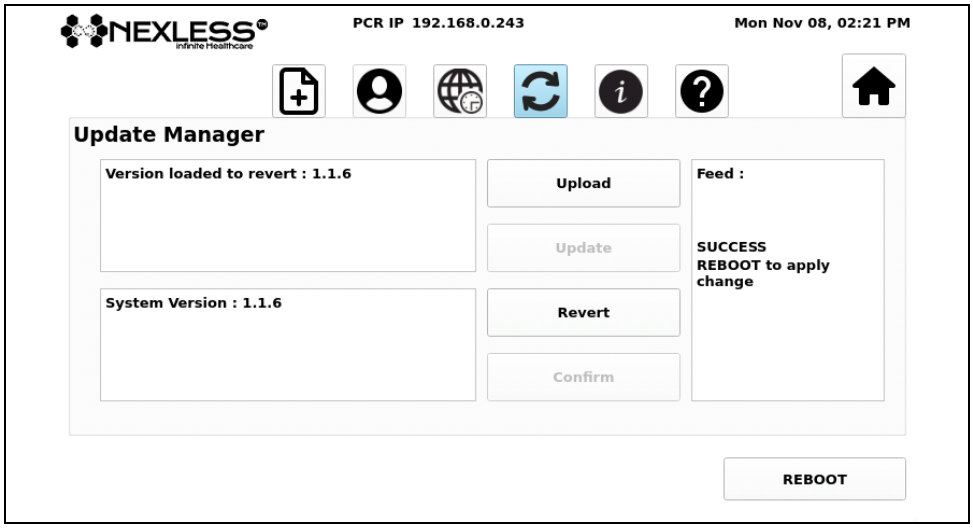


Figure 38 – Reverting to the previous software version

Information

The PCR device internal information can be accessed by clicking the Information icon.

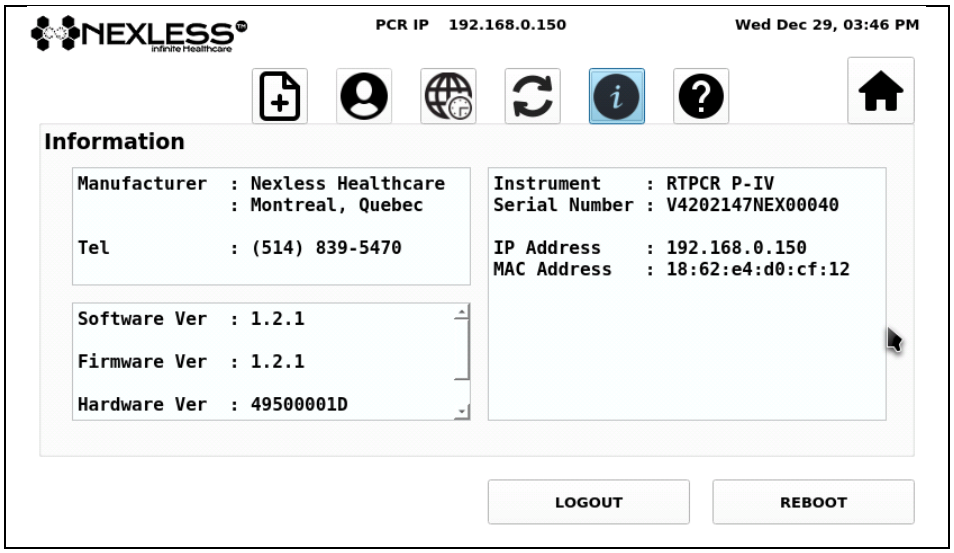


Figure 39 – Information page

Help

The basic help file can be accessed by clicking the Help icon.

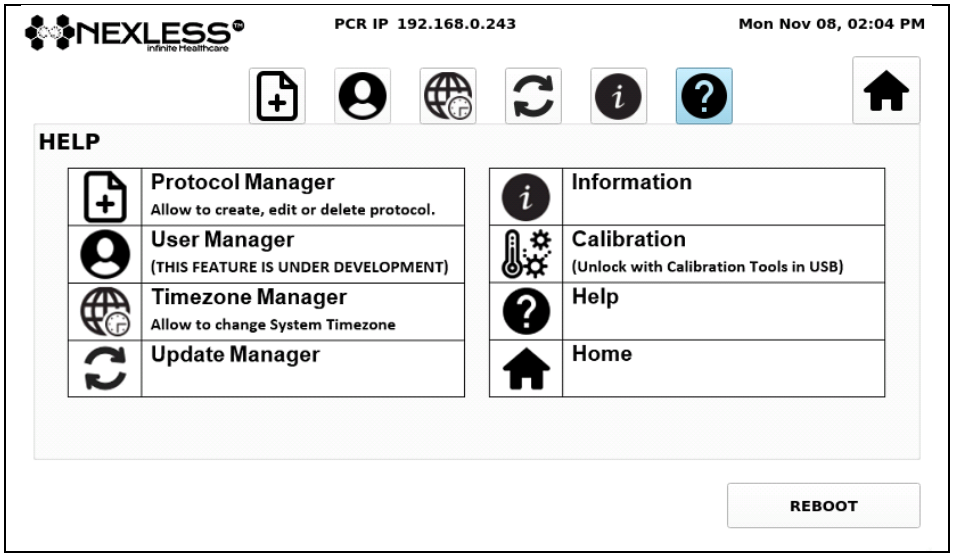


Figure 40 – Help page

Calibration:

The PCR-P4 device temperature sensors are calibrated in factory and will not need to be recalibrated. It has been calibrated using the Progene PCR tubes. If different tubes are used, the device will need recalibration.

Temperature calibration:

Use the Nexless Temperature Calibration Tool to calibrate the device. Before calibration, prepare four tubes with a 20µL solution of D2H2O (19µL) and GNR (1µL) on each tube.

Connect the Temperature Calibration tool to the USB port in the rear of the PCR-P4. The calibration icon will appear on the Settings menu.

Temperature Calibration Icon

Insert the other side of the calibration tool with the 4 thermal sensors in the 4 PCR tubes and into the PCR-P4 device. Close the top Lid and start the calibration.

Automatically, the PCR-P4 will detect the Calibration tool and will display the calibration icon on the settings screen. Click the temperature calibration icon to enter the calibration page. The PCR-P4 device will auto calibrate. Redo the calibration process when different PCR tubes are used.

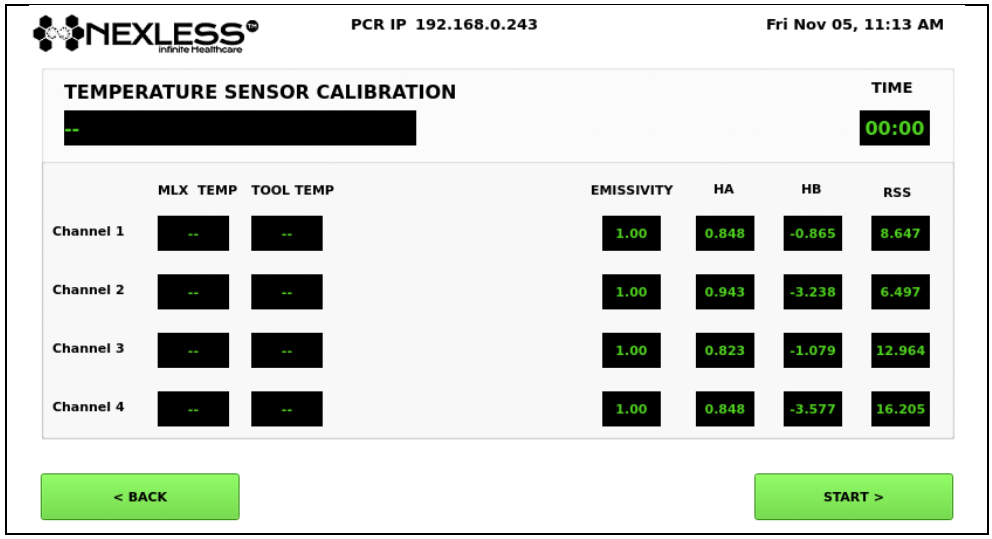


Figure 41 – Calibration page

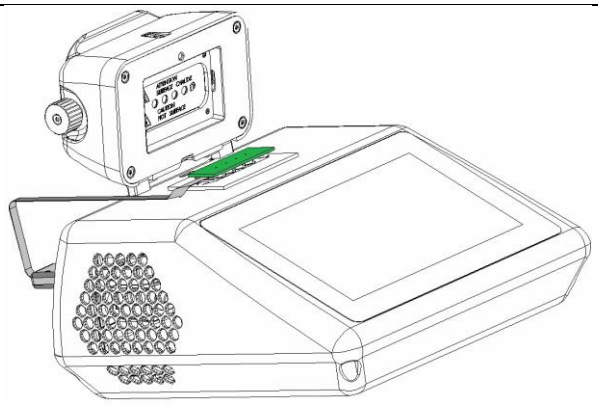


Figure 42 – Inserting the calibration tool and the PCR tubes in the PCR

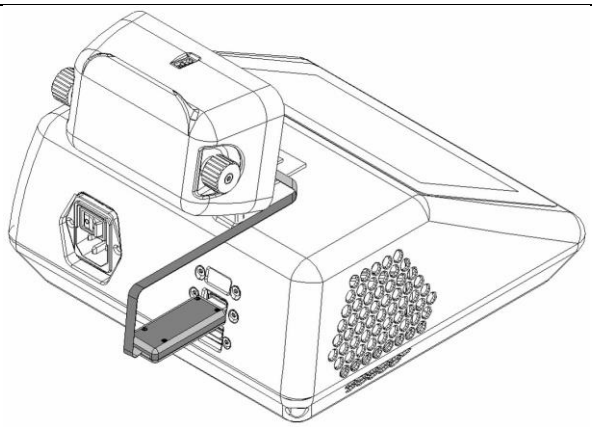


Figure 43 – USB connection of the Calibration tool

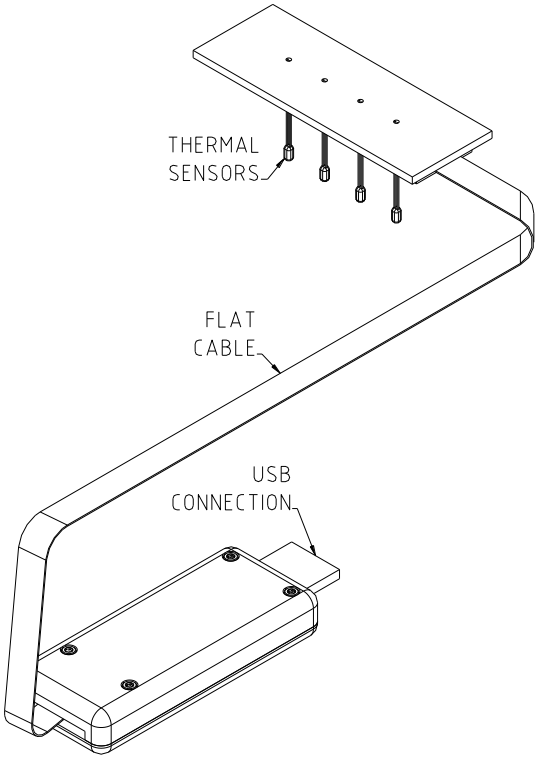


Figure 44 – Calibration tool

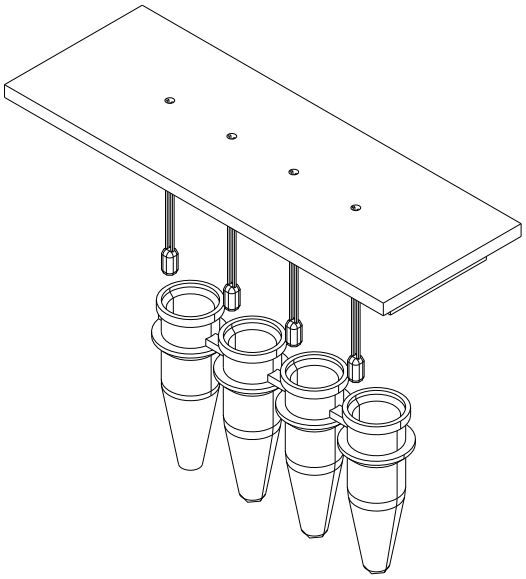


Figure 45 – Calibration tool with the 4 temperature probes in the 4 PCR tubes

Administrative functions

Setting Up an IP Connection with PCR-P4

The device will automatically negotiate an IP address with the DHCP server on the network.

If no DHCP server is found, the device will revert to its default IP address 192.168.27.2. In this case, if you are on Windows, set the IP address of the PC Ethernet port as:

Address = 192.168.27.3
Default gateway = 192.168.27.1
Subnet mask = 255.255.255.0

Connecting the PCR with Windows Explorer

Connecting the PCR to your PC via Windows Explorer is the preferred method of connecting to the PCR. It will help you download the test reports. To connect, please follow these steps:

- Make sure your PC running windows is in the same network as the PCR.
- Open windows explorer.
- In the top left corner, click the “Computer” tab and click on “Map network drive”.
- Note the IP address of the PCR on the front display.
- Enter the folder name “\\192.168.27.2\data” (where 192.168.27.2 is replaced with the actual IP address of the PCR) and check the box labeled “Connect using different credentials”.
- Sign in with the username & password as “pcru” & “pcru” respectively.
- To access the PCR report, Open “**PCR_Run_Tests**” folder. Then open the desired folder. Folders are identified by the date and time of the start of the PCR test.
    - Take note that you should not open the latest folder while the test is still running.
- You can open the test result in your favorite browser by right-clicking on the **output.xml** file and choosing to Open with Microsoft Edge or Google Chrome.
    - Take note, if a graph is not loaded yet, you will need to refresh (F5). It may take up to two minutes to generate all the graphs.
- Once the report is loaded, you can print a copy of the report from your browser.
- We recommend to regularly download all test report directories on your local PC and delete them from the machine.

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