N-Bath User Manual

LIQUID NITROGEN STERILIZING DEVICE

NTERILIZER

Liquid Nitrogen Sterilizing Device

N-Bath User Manual

CE

0425

Revision number: 1.3

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Liquid Nitrogen Sterilizing Device

N-Bath User Manual

For Customer Support, please contact the representative or directly the manufacturer at the following address: info@nterilizer.com

Device name: N-Bath

Version number: 3.0

Manufacturer: Nterilizer SRL, via D'Azeglio 57 - 40123 Bologna Italy

Classification: Medical Device

Table of Contents

N-Bath User Manual

1 Introduction

This User Manual provides information on how to use the Liquid Nitrogen sterilization device, N-Bath. Please ensure that anyone operating this device has read the Manual in its entirety. Keep this Manual handy for future reference. Copying, reproducing or using of any part of this Manual without permission from Nterilizer is prohibited.

1.1 Product Description

1.1.1 Product Name and Purpose

The new Nterilizer N-Bath version 3.0 is an easy-to-operate device designed for the sterilization of Liquid Nitrogen in laboratory, especially for the In-Vitro Fertilization (IVF) procedures of Vitrification and Warming.

1.1.2 Main Characteristics

The N-Bath operates with a sterile disposable sleeve, specifically designed by Nterilizer. The sleeve is equipped with a special label that is sensitive to the Ultraviolet light radiated by the device during sterilization.

The sterilization process, which is traced by a double labelling system, is operated and tracked via the use of dedicated app and portal developed by Nterilizer. More information available at: info@nterilizer.com

The sterilization data is safely uploaded and stored in a web portal based on blockchain technology.

1.2 Warranty and Liability

The following considerations on Warranty and Liability apply to the Nterilizer liquid nitrogen sterilization device, N-Bath. Users shall read and understand this User Manual and observe the safety instructions before operating the device.

Any damage occurring within the period of guarantee will be repaired at no cost. The Warranty is valid within a year of the purchase date.

The warranty is validated if the installation is performed by trained personnel, and the device is used according to the instructions in this Manual.

The Warranty does not include the following conditions:

1. **Damage caused by fire, lightning, improper power supply, and other catastrophic events.**
2. **Damage due to undue adjustments or alterations.**
3. **Damage from improper use or handling.**
4. **Damage from falls or accidents during transportation of the instrument.**
5. **Unauthorized reparations and replacements.**

For assistance, please contact an Nterilizer representative or the manufacturer directly.

1.3 Warnings

The following warnings apply to the Nterilizer Liquid Nitrogen sterilization device, N-Bath.

The main Warnings are:

1. **Read the instructions in this Manual before operating the device.**

2. **Ensure that the device is operated in a certified IVF environment, and only by qualified personnel. The room temperature is to remain between 22 °C and 26 °C, and the humidity between 50 and 70 %.**

3. **Never use this device for different uses, or in different manners from those specified in this Manual.**

4. **Connect the device to a power source that complies with current specifications.**

5. **Wait at least 20 seconds to turn back on the device after it has been turned off.**

6. **Maintenance operations are to be undertaken only by authorized Nterilizer representative's or the manufacturer directly.**

Never attempt to open or modify the device. For any abnormalities, please contact an Nterilizer representative, or the manufacturer directly for technical support.

1.4 Symbols Used in this Manual

The N-Bath device has inherent dangers due to the designated purpose of the product. For safe use of this device, follow the instructions in this Manual and the special indications presented below.

1.4.1 Caution

This symbol indicates that special precaution is needed.

To avoid any risk to the person or instrument, and to follow the intended use of the device, read the text under warning signs carefully.

1.5 Symbols Used in the Device

1.5.1 Symbols on Medical Device Label

Symbol or Acronym	*Description*
REF	Product code
SN	Serial Number
	Manufacturing Date
	Manufacturer Details
	Waste from Electrical and Electronic Equipment and Batteries (RAEE)
	Consult the operating instructions
	Attention, see accompanying documents
	Potential biohazards
	Potential risks from ultraviolet radiation
CE	CE conformity symbol

1.5.1 Electrical Risk

The N-Bath is an electrical device connected to the main power supply. The risk of electrocution incidents exists and can be fatal. As for any electrical appliances, precaution is required especially when dealing with conductive materials such as liquids.

The lid of the device, which houses the power unit, and the base of the device, which provides insulation during sterilization, are not waterproof.

Avoid water or other liquids entering the lid or the base of the N-Bath.

1.5.2 Earth Connection

This symbol indicates earth protection connections. The N-Bath must be connected to a standard and certified electrical socket which includes an effective earthing system.
Earthing minimizes the inherent risks of shock and electrocution that comes with using electrical appliances such as the N-Bath.

1.5.3 Biological Risk

The N-Bath ensures sterilization of Liquid Nitrogen and effective elimination of pathogens and pathological organisms. Special care is needed if the device is used to work with such organisms.

The operator is to be qualified and careful not to be infected from pathogens and pathological organisms. The accessories used for Vitrification or Warming are to be disposed as biohazard waste.

If a contaminated instrument is to be sent back to the Nterilizer representative or directly to the manufacturer for maintenance, it needs to be decontaminated first.

A completed decontamination form is to be included with the shipment documentation. For more information refer to Chapter 4.

1.5.4 Dispose Safely

In order to minimize the waste of electrical and electronic equipment, waste must be disposed in accordance with the Directive 2012/19/EU – *Waste Electrical & Electronic Equipment (WEEE)*. This includes PCBs (lead-free HASL), switches, PC batteries, printed circuit boards and external electrical cables.

All components are in accordance with the RoHS Directive, which states that new electrical and electronic components do not contain lead, mercury, cadmium, hexavalent chromium, polybrominated biphenyls (PBB), or polybrominated diphenyl ethers.

It should, however, be noted that the UV lamps contain toxic compounds including mercury, irrespective of its physical condition. This is in accordance with the provisions of the RoHS Directive mentioned above.
Considering the toxic content, the UV lamps should be disposed of according to local waste management requirements and environmental legislation.

They should not be opened or burned since they may emit toxic fumes. For more information on disposal refer to Chapter 4.

1.5.5 CE Mark

CE
0425

CE Mark representative: Nterilizer SRL Via D'azeglio, 57 - 40123 Bologna Italy
Tel. +39 335 330 836 | E-mail. info@nterilizer.com

The mark "CE" originated in 1985 as an abbreviation of Conformité Européenne (French for European Conformity).

CE marking indicates conformity with EU directives regarding health, safety, and environmental protection standards for products sold within and outside of the European

Economic Area.

A copy of conformity declaration for the Nterilizer sterilization device N-Bath is included in Chapter 9.

1.6 Intended Use

The intended use of the Nterilizer device N-Bath is to provide an efficient and effective method for sterilizing Liquid Nitrogen to be used in a certified IVF environment. Nterilizer has undertaken a number of international and peer reviewed tests, evaluations, and publications, demonstrating the effectiveness and efficieny of its technology (for more information visit the *Bibliography* section on the Nterilizer Website).

The N-Bath utilises UV light radiation of type C as a successul and easy to implement method for:

- Preparation of Sterile Liquid Nitrogen for the procedures of Vitrification.
- Preparation of Sterile Liquid Nitrogen for the procedures of Warming.

The device can also be used for other applications including cryopreservation of cell/tissue, cryosurgery, etc.

This Manual is only referring to the following uses in certified IVF environment: Vitrification and Warming.

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2 Device Overview

The N-Bath is designed for the Vitrification of biological specimes with sterile Liquid Nitrogen, as well as for the Warming of samples stored in Liquid Nitrogen.

The three main components of the N-Bath are (Figure1):

- The **N-Lid:** a removable lid, made of ABS, that fits over the insulation case and which houses the power unit, the Ultraviolet (UV) lamps, the unit controller and the bluetooth system.
- The **N-Tub**: a removable tub made of stainless steel which fits into the insulation box.
- The **N-Case**: an insulation case made of Acrylonitrile Butadiene Styrene (ABS) on the outside, and high density polystyrene on the inside, which prevents Liquid Nitrogen from evaporating during the sterliziation phase.

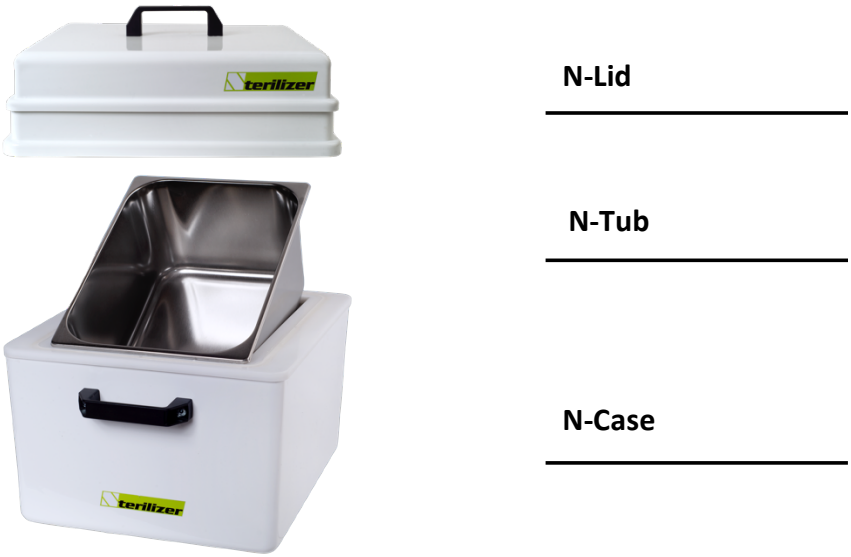

Figure 1. Main components of the Nterilizer device N-Bath

The N-Bath is designed to increase sterilization effeciency and minimize the loss of Liquid Nitrogen through evaporation and the risks arising from the presence of Liquid Nitrogen. For more details on the safety of using Liquid Nitrogen please refer to section 3.3.

The devise is also designed to block the dispersion of UV light.

The N-Bath is designed to operate wirelessly in conjunction with the two following accessories provided by Nterilizer (Figure 2):

- The **N-Controller**: a tablet connected to the N-Bath via Bluetooth technology which is used to control the sterilization procecces.
- The **N-Printer**: a printer connected to the N-Controller via Bluetooth technology which is used to print labels certifying the completed sterilization proceses.

Figure 2. Wireless accessories provided by Nterilizer to operate with the N-Bath

The N-Bath and the N-Printer are operated with the N-Controller via a dedicated application provided by Nterilizer, the **N-App** (Figure 3). The N-App allows the user to set-up the sterilization process, safely start and stop the device, and record data for each sterilization event. For more details on how to operate the N-App, the N-Controller, and the N-Printer see Chapter 3 and APPENDIX 1.

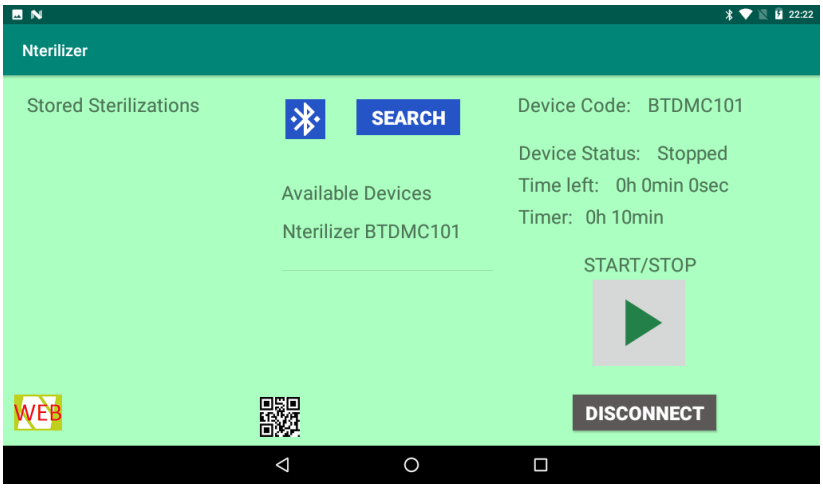

Figure 3. Screen view of the N-App, provided by Nterilizer to operate the N-Bath

The N-Bath is designed to operate in conjunction with three accessories recommended by Nterilizer (Figures 4 and 5). These are briefly described below (for more details refer to Chapter 3):

- The **N-Box**: a reusable box, made of polystyrene with high thermal characteristics, which is to be placed inside the stainless steel tub.
- The **N-Sleeve:** a sterile disposable sleeve, made of medical-grade Ethylene Vinyl Acetate (EVA) which is to be placed inside the N-Box and filled right up to the top with the Liquid Nitrogen to be sterilized for Vitrification or Warming. The sleeve comes with a UV light-sensitive detachable label (see circled in Figure 4) wich turns from yellow into green when sufficient radiation is detected.
- The **N-Test:** a microbiological kit which is used to test the effectiveness of the UV lamps (see Figure 5. For more details refer to APPENDIX II).

Sterilization takes place by exposing for a precise amount of time the amount of Liquid Nitrogen contained inside the N-Sleeve to a UV light of type C, with 254 nm of wavelength. The sterilization time is automaticaly calculated by the unit controller housed inside the lid to adapt to environmental conditions, increasing or reducing so to ensure the effectiveness of the sterilization.

The bluetooth technology enables operating the device in an effective and simplified manner. The N-App, in conjunction with the N-Controller and N-Printer, allows to fully control the sterilization process and printing operations, as well to change settings, safely start and stop the device, and record data for each sterilization event. For more details see Chapter 3 and APPENDIX I.

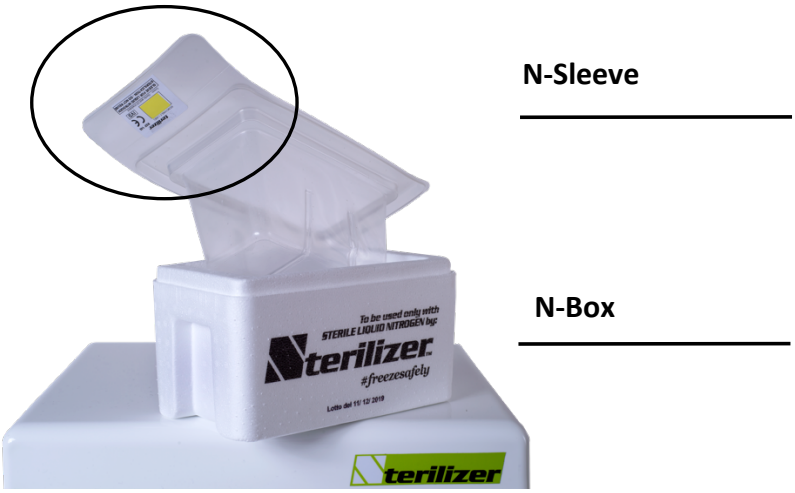

Figure 4. N-Bath recommended accessories N-Box and N-Sleeve. Highlighted in the circle is the UV light-sensitive detachable label.

Figure 5. N-Bath recommended accessory N-Test

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3 Operating Procedure

3.1 Quick Start Guide

A schematic representation of the N-Bath operating procedure is presented below (Figure 6).

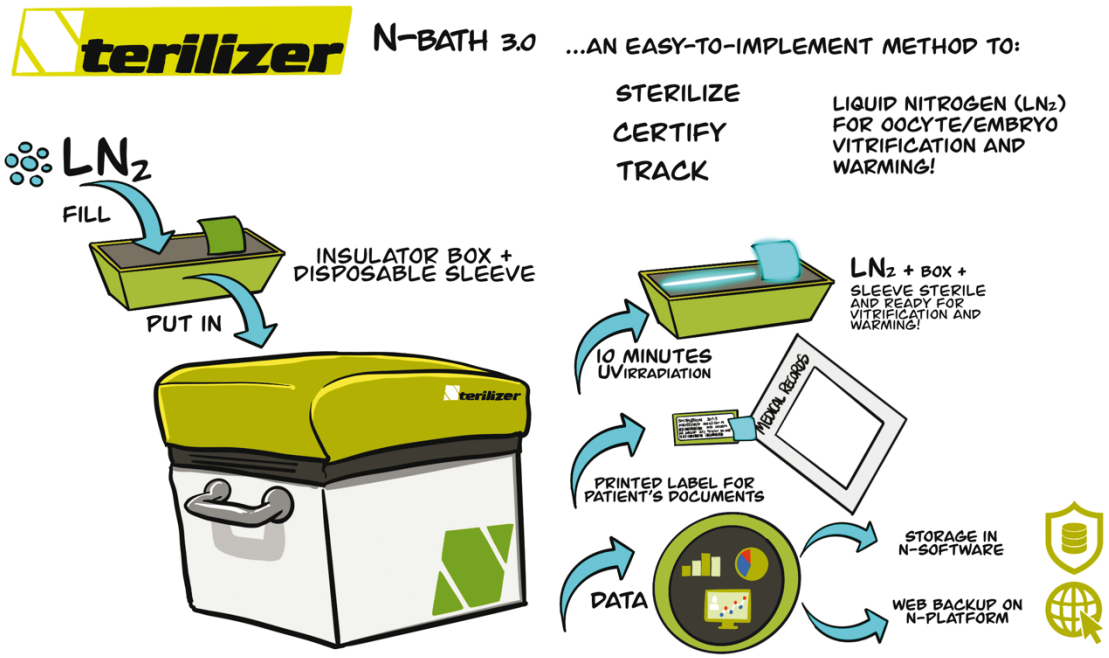

Figure 6. Overall procedure for operating the N-Bath

The main operating steps are:

1. Remove the cover (N-Lid) and place it on a stable surface.
2. Ensure the stainless-steel tub (N-Tub) is correctly fit in place inside the N-Case.
3. Apply a new sterile N-Sleeve inside the N-Box; ensure the UV Light sensitive label on the sleeve is yellow (indicating that it has not been used previously).
4. Place the N-Box inside the N-Tub; ensure that the N-Sleeve is still correctly in place.

5. Fill the N-Sleeve right up to the top with fresh Liquid Nitrogen. (For more details on the safety of using Liquid Nitrogen please refer to section 3.3).

6. Close the N-Lid ensuring it is correctly in place over the N-Case.

7. Ensure that the device and the printer (N-Printer) are connected to a power source, and the wi-fi network is working correctly.

8. Turn on the device with the switch located on the N-Lid.

9. Turn on the tablet (N-Controller), launch the Nterilizer application (N-App) and ensure that the device is connected via Bluetooth (note for the message 'Available Devices', refer to APPENDIX I).

10. Start the sterilization process by pressing the bottom 'Start/Stop'. An algorithm will automatically calculate the duration of the sterilization process, depending on environmental conditions. Generally, the sterilization will last for approximatively 10 to 11 minutes. If for whatever reason the sterlization has to be stopped before it is completed, it is sufficient to press the bottom 'Start/Stop' again.

11. Once the sterilization process is finished, the message 'Time Left 0h 0min 0sec' will appear on the tablet (Look Picture 6 in APPENDIX I). Open the N-Lid and check that the UV light sensitive label located on the sleeve has turned to green (indicating that sufficient radiaiton was detected).

12. Print the label via the N-App to confirm the sterilization process, and to provide a certified record toghether with all the data.

13. Place the label into the patient book together with the label that you would have printed (step 11).

14. Use the App to send the sterilization data to the Nterilizer web platform.

15. You are now provided with certified sterilized Liquid Nitrogen and you can safely begin Vitrification or Warming. (For more details on the safety of using Liquid Nitrogen please refer to section 3.3).

16. When finished with the Vitrification or Warming process, dispose of the Liquid Nitrogen according to your standard procedures, and the sleeve according to the disposal suggestions provided on Chapter 4.

17. You can safely re-use the N-Box for another Vitrification or Warming (provided no biological contamination has occurred) up to a recommended total of no more than 50 cycles. Before a new use ensure that the N-Box has been thoroughly cleaned and dried. Apply a new sterile N-Sleeve each time.

For further details refer to the Detailed Procedure presented below and Chapter 6, Common Issues.

For any issues during the device operation, please contact Nterilizer representative or the manufacturer directly for technical support.

3.2 Detailed Procedure

Controls are performed through the N-Tablet via the N-App. The app allows the user to safely Start and Stop the device.

 The **'Start/Stop'** button starts the timer and gives confirmation when selecting.

 The **'Start/Stop'** button also stops the timer, if necessary, and gives confirmation when selecting.

The device utilises two special lamps which deliver suitable UV light of type C (UVC). The standard duration for the sterilization process is + or - 10 minutes, corresponding to the results of research validated by peer review tests undertaken by Nterilizer (available on the 'Bibliography' section of Nterilizer website).

The device automatically adjusts the duration of the sterilization process according to environmental conditions. At the start the system will show a significantly longer time because the lamps are at room temperature and therefore the intended performance will be lower. Gradually, as the temperature of the lamps reaches the working level, the time will be reduced. This feature ensures results regardless of the environmental conditions (if the air temperature is particularly low, the time would be automatically extended to expose Liquid Nitrogen to the needed amount of UVC). The typical duration of the sterilization process ranges between 10 and 11 minutes

After approximatively 10 minutes of sterilization it is ensured that UV light exposure of 660,000 units is more than adequate for the most resistant microorganisms, as per internationally peer reviewed UV table (available on the 'Bibliography' section of Nterilizer Website).

The effectiveness of the sterilization depends on the condition of the UV lamps.

The UV lamps need to be tested regularly using the Nterilizer kit N-Test. For more information refer to APPENDIX II.

The timer stops when the remaining time is zero. The message 'Time Left 0h 0min 0sec' (refer to APPENDIX I) appears confirming that the sterilization was successfully completed. It is now safe to open the N-Lid and check that the UV light sensitive label located on the sleeve has turned to green, indicating that sufficient radiaiton was detected (Figure 7).

Figure 7. The UV light-sensitive detachable label located on the N-Sleeve is yellow before radiation (left photo) and becomes green after radiation (right photo)

The N-Printer provided by Nterilizer is designed to produce a record of the sterilization process. The printer, which can be operated by the tablet via the N-App, allows the user to print a sterilization label which contains all informaiton about the completed process, including the code of the patient (or the code of the carrier in case of Warming procedure), the delivered radiation, and the duration.

Once printed, this label is to be placed into the patient book, together with the green label from the N-Sleeve. To complete the certification procedure, the sterilization data is to be sent via the N-App to the Nterilizer web platform.

It is now safe to use the certified sterilized Liquid Nitrogen for Vitrification or Warming procedures. (For more details on the safety of using Liquid Nitrogen please refer to section 3.3). Once the Vitrification or Warming process is complete, the Liquid Nitrogen can be disposed, according to the procedures endorsed by the clinic. The sleeve is one use only and is to be disposed according to the suggestions provided on Chapter 4.

In case of no contamination, the N-Box may be reused for up to 50 cycles, and then be disposed according to the suggestions provided on Chapter 4. If the N-Box is to be reused, it is recommended that it is thoroughly cleaned and dried before a new sterile N-Sleeve is applied.

If contamination occurred during the Vitrification or Warming, the N-Box cannot be reused and is to be disposed according the suggestions provided on Chapter 4.

3.3 Liquid Nitrogen Safety

Liquid Nitrogen appears as a colourless liquid and temperatures between about -210°C and -195°C. The main risks of Liquid Nitrogen are: 1. contact of parts of the body with Liquid Nitrogen or Nitrogen vapour, which is at the same temperature, as it can cause severe cold burns and, if prolonged, can lead to freezing of the affected part; 2. reduction of the amount of oxygen in the ambient air due to Nitrogen vapours; 3. exposure to physical agents deriving from the use of Liquid Nitrogen.

Special attention is therefore required by the operators in order to reduce the main risks due to the handling of Liquid Nitrogen. For specific procedures for the safe use and storage of Liquid Nitrogen, Nterilizer refers operators to the industry standards in force, and the procedures adopted by the laboratory in which the N-Bath device is intended to be used.

For informational purposes, the following are standard safety measures for the use of Liquid Nitrogen.

Before using Liquid Nitrogen, ensure that you are familiar with the contents of the Material Safety Data Sheet (or Material Safety Data Sheet) of Liquid Nitrogen, carry out an analysis of the environmental safety conditions, check that there is sufficient ventilation, and that the air monitoring devices and alarm systems are active. It is recommended that you do not operate alone or take other countermeasures to avoid the danger of asphyxiation when using the medical device in a confined environment, such as installing oxygen sensors.

Particular attention should be paid to the operations of transferring and the operations of immersion and extraction of objects from Liquid Nitrogen, due to the production of splashes due to the temperature change of Liquid Nitrogen. These operations must be carried out slowly and include the use of Personal Protective Equipment (PPE) in accordance with the law, such as: gloves to protect against cold burns, goggles with side protection or visors and lower limbs protection to prevent dripping inside the footwear, especially during decanting operations. Gloves must be wide so that they can be easily removed if drops or splashes enter them.

Safety procedures are required when handling Liquid Nitrogen, as specified by industry standards, which includes wearing suitable clothing, special gloves, and a special screen to protect the face.

For safety, it is recommended to place the N-Bath on a stable surface. Tilting the device, even momentarily, can cause a spill of Liquid Nitrogen outside of the N-Box leading to risk of harming the operator.

When removed, the lid is to be placed on a safe surface adjacent to the device.

The cover is made of fragile parts, such as the special glass that guarantees the system isolation, so it's breakage can cause damage and danger to the operator.

Before filling the N-Sleeve with Liquid Nitrogen ensure that the device is powered off and the lid is securely placed.

With the use of a spiller, fill right up to the top N-Sleeve with Liquid Nitrogen, as shown in the figure below (Figure 8).

During filling operation, use extreme care.

Avoid any Liquid Nitrogen spills as it can damage the eyes and the skin and cause severe burns.

When the Liquid Nitrogen (-196 ° C) meets surfaces that are at room temperature, it will boil and create considerable amount of vapour emissions due to humidity condensation in the air.

Ensure that the area is sufficiently ventilated to handle the vapour generated by Liquid Nitrogen.

LN to be filled up to the top of N-Sleeve

Figure 8. Filling the N-Sleeve with Liquid Nitrogen inside the N-Sleeve

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4 Maintenance Procedure

4.1 Parts Duration and Disposal

4.1.1 N-Box and N-Sleeve

The N-Box can be washed and reused for several times. The N-Sleeve cannot be reused and is to be treated as contaminated material when disposing.

The N-Box has a life duration of 50 uses before it requires replacing. The N-Sleeve is only to be used once.

4.1.2 Liquid Nitrogen

Liquid Nitrogen must be disposed of according to the regulations in force and the procedures adopted by the laboratory. For more details on the safety of using Liquid Nitrogen please refer to section 3.3.

4.1.3 UV Lamps

Lamps Description

The two UV lamps used by the Nterilizer technology, which are housed inside the lid of the N-Bath (Figure 9), are special lamps with specifications that meet the requirements for an effective and efficient sterilization of Liquid Nitrogen. These lamps contain toxic compounds including mercury, irrespective of its physical condition. This is in accordance with the provisions of the RoHS Directive mentioned in Chapter 1.

Attempts to remove the lamps could damage the device and pose risks to the operator.

The screen covering the UV lamps should never be opened by a non-qualified operator.

Figure 9. UV lamps used are housed inside the lid of the N-Bath

Lamps Operations

The UV lamps operate correctly in a limited temperature range, so a sensor automatically verifies that the temperature is not too low or too high. In the case of low temperature, the system alerts the user with the message, 'Probe Error', which means the temperature is too low or too high.

Lamps Control

In case one or both lamps are defective or do not function correctly, the device will detect a malfunction and display the message 'Lamp Error'. For more details refer to Chapter 6.

Lamps Testing

To ensure the lamps are still functioning as expected, and guarantee the maximum level of sterilization, regular testing is required with the use of the dedicated kit, N-Test.

Do not attempt to replace the lamps as it could cause injuries to the operator or damage the device. Contact the representative or the manufacturer in case the N-Test reveals an anomaly, indicating that the lamp's life is coming to an end.

The N-Bath lamps need to be tested regularly, using the dedicated N-Test. For more information refer to APPENDIX II.

4.2 Cleaning

Cleaning the unit requires that all parts have returned to room temperature. It is necessary to unplug the power cord and separate the various parts: the lid, the steel tub and the insulating box.

Each part must be cleaned thoroughly using suitable products used for cleaning laboratory equipment. A solution with 70% ethanol can be safely used.

Never use corrosive substances such as mineral spirits, iodine, hydrochloric acid, and sulphuric acid for cleaning the device.

Corrosive cleaning products can damage the N-Bath and are dangerous for the operator.

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5. Specifications, Transport and Storage

5.1 Specifications

Model	N-Bath 3.0
Exterior Dimensions	Length 500 (mm) x Depth 370 (mm) x Height 490 (mm)
Exterior Material	Plate ABS
Interior Material	Stainless steel sheet
Thermal Insulation	Polystyrene
Power supply	AC230V 50Hz 0,4A (max)
Fuses	2 x 5x20 glass 0,4 AT
Lamps used	Philips TUV-PL-S 9W, Osram HNS S 9 W or equivalent
Weight	14 kg
Operating Environment	22 °C – 28 °C
Altitude	< 2000 m
Installation Category	I
Acoustic Level	The device operates well below the level required by ISO 3746 or ISO 9614-1
Shipping dimensions	Width 555 mm x Depth 480 mm x Height 585

5.2 Transport and Storage

Nterilizer recommend transporting the device using the original package, specially designed by Nterilizer. Provide a storage temperature range between 5° C and 45° C with humidity not above 80%.

The packaging has been specifically designed to guarantee a correct management of the transport phase of the N-Bath device and is composed by:

- External box made of double wave corrugated cardboard, which guarantees the level of resistance considered adequate for the correct transport of the device (Figure 10);
- Protective shells made of preformed shockproof polyethylene foam (Figure 11).

Figura 10. Scatola esterna in cartone ondulato

Figura 11. Gusci protettivi in foam preformato

The labelling on the packaging shall contain the following information:

- Symbols for correct handling during transport and storage (orientation of the package, fragile content, protection from water, minimum and maximum temperature and humidity value);
- Content identification elements in accordance with the labelling requirements of Directive 93/42/EEC.

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6 Common Issues

The most common issues and solutions are presented in the table below**.**

Problem Detected	Monitoring and solutions
No Available Device	**1.** Check that the N-Bath is properly connected to a power outlet and that the switch is turned on. **2.** Check the fuses and replace if necessary (to be undertaken by certified technician only). **3.** If the issue is still unresolved, call for technical support.
Message Probe Error	**4.** Check that the level of Liquid Nitrogen is correct. **5.** Make sure that the room temperature is not too cold (a suitable environment for operating the N-Bath is 22-26 ° C). If necessary, wait for it to be at the right temperature. **6.** If the issue is still unresolved, call for technical support.
Message Lamp Error	**7.** Verify that the N-Lid is correctly positioned over the N-Tub. **8.** If the issue is unresolved, call for technical support.

This table is only a guide for resolving the most typical issues.

For assistance with any other issues please contact the Nterilizer representative or the manufacturer directly.

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7 Accessories and Spare Parts

7.1 Accessories

Component	Details	ID Code
N-Test	Microbiological test kit for UV lamps	M000003
N-Box	Polystyrene box for Vitrification and Warming	M000001
N-Sleeve	Disposable EVA sleeve to apply inside the N-Box	M000002

For the correct use of the N-Bath accessories please refer to the specific sections in this Manual (for N-Test refer to APPENDIX II).

For assistance please contact the Nterilizer representative or manufacturer directly.

7.2 Spare Parts

Component	Details	ID Code
Fuses	2 x 5x20 glass 0,4 AT	R000026
Lamps	Philips TUV-PL-S 9W, Osram HNS S 9 W or equivalent	E000017

Maintenance of N-Bath and parts replacement is to be operated by a certified technician only.

Please contact the Nterilizer representative or the manufacturer directly for assistance.

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8 Operating Protocols

8.1 Liquid Nitrogen Sterilization Prior to Vitrification

PROTOCOL		
NAME: Liquid Nitrogen Sterilization Prior to Vitrification		DEVICE: N-Bath 3.0.
UTILITY: This protocol is designed for the preparation of Sterile Liquid Nitrogen (LN_2) for the procedure of Vitrification, and to ensure that the Vitrification process is carried out under certified sterile conditions. For specific Vitrification procedures refer to your clinic protocols.		
PROTOCOL ID: N-Bath_V	VERSION: 1.0	DATE: 24 October 2019

STEP	DESCRIPTION	NOTES
1	Place the device inside the sterile cabinet where you are planning to perform the Vitrification procedure. Ensure the device is powered off.	⚠ Note A
2	Remove the cover (N-Lid) and place it on a stable surface.	
3	Ensure the stainless steel tub (N-Tub) is correctly fit in place inside the N-Case.	
4	Apply a new sterile N-Sleeve inside the N-Box; ensure the UV Light sensitive label on the sleeve is yellow (indicating that it has not been used previously).	
5	Place the N-Box inside the N-Tub. Check that the N-Sleeve is still correctly in place inside the N-Box.	
6	Fill right up to the top the N-Sleeve with fresh LN_2.	⚠ Note B
7	Close the lid. Connect the device to power.	
8	Ensure that the device and the printer (N-Printer) are connected to the power source, and the wi-fi network is working correctly.	
9	Turn on the device with the switch located on the N-Lid.	
10	Turn on the tablet (N-Controller), launch the Nterilizer application (N-App) and ensure that the device is connected via Bluetooth (note for the message 'Available Devices').	
11	Start the sterilization process by pressing the bottom 'Start/Stop'. An algorithm will automatically calculate the duration of the sterilization process, depending on environmental conditions.	

	Generally, the sterilization will last for approximatively 10 to 11 minutes. If for whatever reason the sterilization has to be stopped before it is completed, it is sufficient to press the bottom 'Start/Stop' again.	
12	When sterilization is complete the time counter on the N-Controller stops. Remove the lid and place it on a stable surface. Check that the UV light sensitive label has turned green indicating that sufficient radiation was detected. Remove the N-Box from the N-Tub and place it where you will perform the Vitrification procedure.	⚠ Note C
13	Add information on the label as needed and save the modification.	
14	Print the label via the N-App to confirm the sterilization process, and to provide a certified record toghether with all the data.	
15	Place the label into the patient book together with the label that you would have printed (step 14).	
16	You are now provided with certified sterilized LN_2 and you can safely begin Vitrification.	
17	Use the N-App to send the sterilization data to the Nterilizer web platform.	
18	When finished with the Vitrification process, dispose of the Liquid Nitrogen according to your standard procedures, and the sleeve according to the disposal suggestions provided on Chapter 4.	
19	You can safely re-use the N-Box for another Vitrification, provided no biological contamination has occurred, up to a recommended total of no more than 50 cycles. Before a new use ensure that the N-Box has been thoroughly cleaned and dried. Apply each time a new sterile N-Sleeve	
20	Repeat the procedure from Step 1 for each patient.	⚠ Note D

NOTES	
A	If your sterile cabinet is not large enough to house the device, place it as close as possible to the cabinet; in this event, sterility for the entire Vitrification procedure cannot be certified.
B	Use safety procedures for handling LN_2. Medical-grade LN_2 is strongly recommended.
C	Use the App to download the data and print a label with all sterilization details (patient code, operator, etc); add to this label the removable label from the disposable sleeve.
D	Use the App to send the sterilization data to the Nterilizer web platform.

8.2 Liquid Nitrogen Sterilization Prior to Warming

PROTOCOL		
NAME: Liquid Nitrogen Sterilization Prior to Warming		DEVICE: N-Bath 3.0.
UTILITY: This protocol is designed for the preparation of Sterile Liquid Nitrogen (LN_2) for the procedure of Warming, and to ensure that the Warming process is carried out under certified sterile conditions. The protocol is recommended for any Warming procedure, both if the carrier was prepared and stored with sterile LN_2 or if it was vitrified/prepared/stored in non-sterile LN_2. For the specific Warming procedures refer to your clinic protocols.		
PROTOCOL ID: N-Bath_W	VERSION: 1.0	DATE: 24 October 2019

STEP	DESCRIPTION	NOTES
1	Place the device inside the sterile cabinet where you are planning to perform the Warming procedure. Ensure the device is powered off.	⚠ Note A
2	Take a new Warming box; if reusing one ensure that it is thoroughly clean and dry. Fit a new disposable sleeve inside the box.	
3	Open the device lid and place the box inside the stainless-steel tub. Check that the sleeve is still correctly in place inside the box.	
4	Fill right up to the top the N-Sleeve with fresh LN_2 that has not been in contact with any biological specimens.	⚠ Note B
5	Close the lid. Connect the device to power and switch it on.	
6	Follow the instructions on the App to initiate the sterilization process.	
7	When sterilization is complete, remove the lid and place it on a soft surface. Check that the UV light sensitive label has turned green. Remove the box from the tub and place it where you will perform the Warming procedure.	⚠ Note C
8	Extract the first carrier from the storage dewar and quickly immerse the carrier in the sterilized LN_2. Unseal it or open the cap and swirl the carrier back and forth at least 3 times (3 washes) and for about 30”; ensure the carrier remains submerged in LN_2 during this process. Remove the carrier from the LN_2 and immerse it in Warming solution.	
9	Repeat the procedure from Step 1 for each carrier.	⚠ Note D

NOTES	
A	If your sterile cabinet is not large enough to house the device, place it as close as possible to the cabinet; in this event, sterility for the entire Warming procedure cannot be certified.
B	Use safety procedures for handling LN_2. Medical-grade LN_2 is strongly recommended.
C	Use the App to download the data and print a label with all sterilization details (patient/carrier code, operator, etc.); add to this label the removable label from the disposable sleeve.
D	Use the App to send the sterilization data to the Nterilizer web platform.

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9 Certifications

9.1 CE Conformity Declaration

N-Bath is a Class IIA medical device according to Regulation 15 of Annex IX of European Directive 93/42/EC and complies with the following European Directives:

- **Directive 93/42/EEC** of 14 June 1993 concerning medical devices, published in the Official Journal of the European Community N.L. 169 of 12/07/1993, and transposed in Italy with D.Lvo n° 46 of 24 February 1997 and relati s.m.i.
- **Directive 2014/35/EU** relating to electrical equipment designed for use within certain voltage limits.
- **Directive 2014/30/EU** relating to electromagnetic compatibility.
- **Directive 2014/53/EU (RED)** relating to making available on the market of radio equipment.
- **Directive 2011/65/EU (RoHS)** relating to the restriction of the use of certain hazardous substances in electrical and electronic equipment.

9.2 Certifications Available Upon Request

- N-Bath Microbiology certification.
- N-Bath UV certification.

If a certification is required, please contact your representative or the manufacturer.

N-Bath User Manual

APPENDIX I. N-Controller and N-App detailed procedures

Part A. Scan a New Batch of N-Sleeves

This section provides a step-by-step description of how to record the unique number of each batch of disposable N-Sleeves before proceeding with sterilization processes.

Before initiating a new sterilization for Vitrification or Warming, it is necessary to ensure that the batch of disposables N-Sleeves that is intended to use is correctly recorded. Without this step, which is necessary for quality control and tracking, is it not possible to proceed with sterilization.

1. Turn on the N-Controller for the first time

Turn on the N-Controller by using the switch located on the side of the tablet. If it is the first time the N-Controller is turned on by the user, a 'QR CODE WARNING' window appears (Figure 1), inviting the user to scan the bath of N-Sleeves that is indented to use for sterilization.

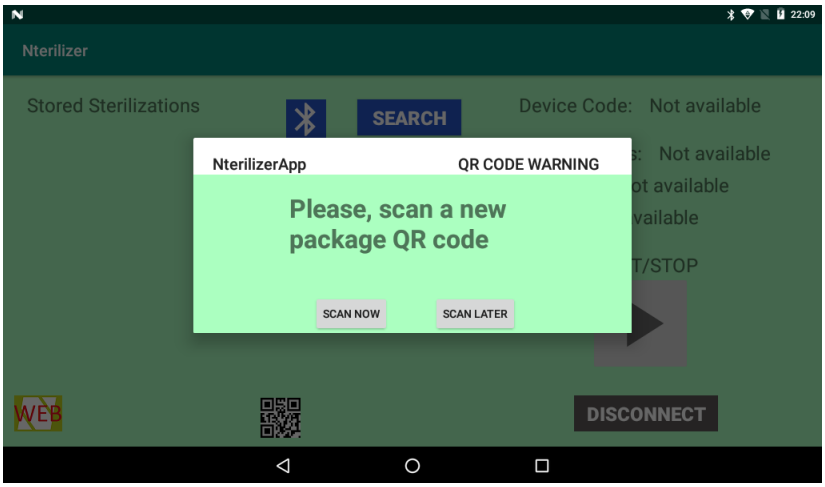

Figure 1. Screen view when the N-Controller is turned on for the first time

The user has the option to initiate the scan procedure, by selecting 'SCAN NOW', or delay this process, by selecting 'SCAN LATER'. The former option will enable sterilization, while the latter will block it.

2. Scan the first batch of N-Sleeves

If 'SCAN NOW' is selected, the user will be invited to point the N-Controller's camera to the QR code of the N-Sleeve's batch that is intended to use, as shown in Figure 2.

Figure 2. Use the N-Controller's camera to scan the QR code of a new batch of N-Sleeves

Once the QR code is read and automatically saved, the N-App unblocks the sterilization process and returns to main screen (Figure 3).

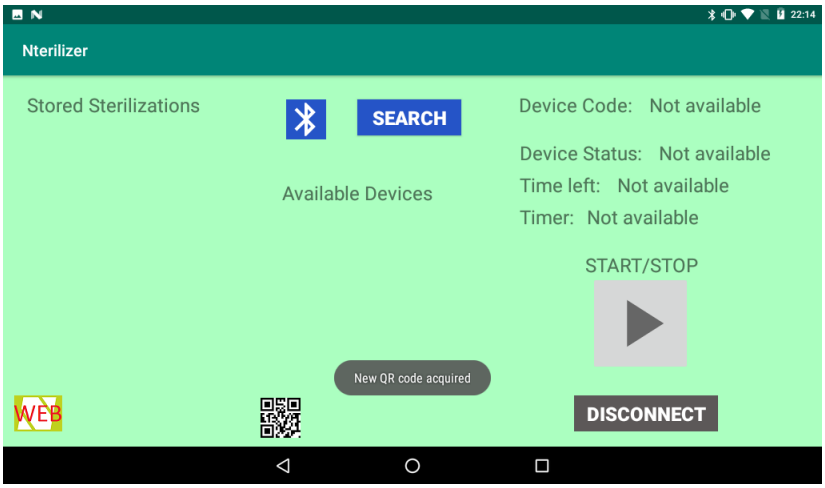

Figure 3. The QR code is correctly saved, and the sterilization process can initiate

The N-App has an in-built counter which monitors how many completed sterilizations are undertaken with each batch of N-Sleeves.

Once a new QR code is saved, the system allows for a maximum of ten successful sterilizations, after which the 'QR CODE WARNING' window shown in Figure 1 appears, indicating that a new QR code is requested.

3. Scan a new batch of N-Sleeves

The user can choose to scan a new batch of N-Sleeves ('SCAN NOW') or postpone ('SCAN LATER'). Unlike for Step 1, if the user decides to defer it is now possible to proceed with new sterilizations using the existing QR code.

Note: If the user chooses not to scan the QR code of a new batch of N-Sleeves, all new sterilizations will be associated with the last saved QR code (referring to the previous batch of N-Sleeves). This information will be saved in the user's portal, together with the rest of the information pertaining the sterilization process, and a notification will be sent to Nterilizer.

Every time a new QR code is scanned, the previous batch number will be replaced by the new one, and the sterilization counter reset to zero. The user can also choose to scan a new QR code before receiving the 'QR CODE WARNING' notification. To do this, it is sufficient to tap the QR code-shaped icon (QR code Reader) which will initiate a new scanning process. If the batch currently in use is accidentally scanned again before reaching the end of endorsed sterilizations, the system will recognise this and avoid resetting the counter.

Part B. Initiate the Sterilization

This section provides a step-by-step description of how to use the N-Controller and the Nterilizer dedicated app (N-App) to operate the N-Bath for the procedures of Vitrification and Warming. Refer to the Manual for a description of the procedures for operating the N-Bath.

1. Preparation

Turn on the N-Bath and the N-Printer, ensuring they are correctly connected to power. Turn on the N-Controller by using the switch located on the side of the tablet (the screen shown in Figure 1 appears). Swipe up with one finger (the screen shown in Figure 2 appears).

Figure 1. Screen view when the N-Controller is turned on

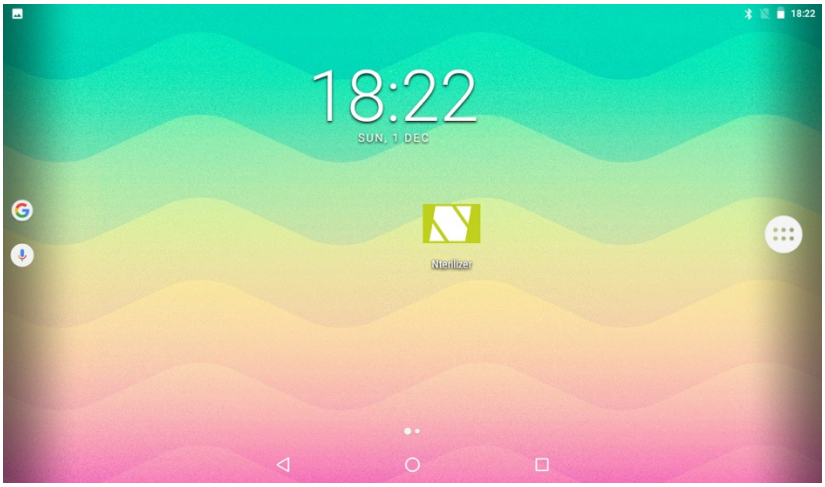

Figure 2. The Nterilizer app appears on the screen

2. Connect the device

Tap on the Nterilizer icon to launch the N-App and connect to the N-Bath. The following screen appears, indicating that the N-Controller is trying to connect to the device (Figure 3).

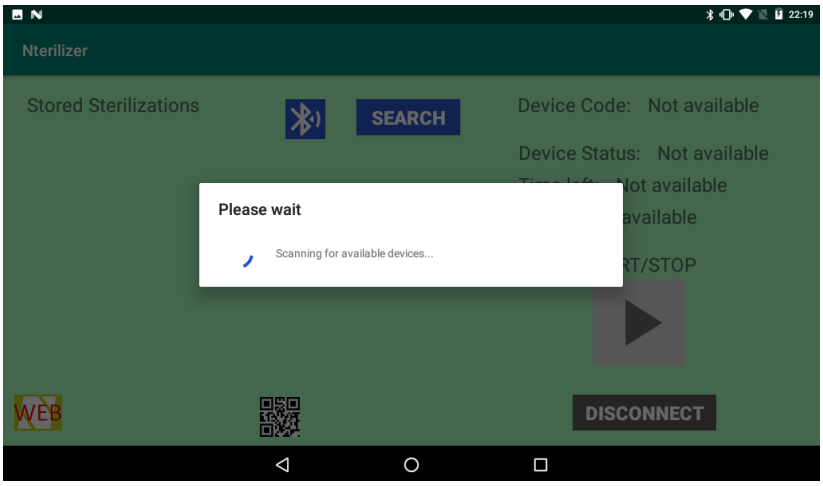

Figure 3. The N-Controller is trying to connect to the N-Bath

If the N-Bath is taking longer to connect, check the device to ensure it is correctly connected to power and that it is located ideally within 5 m from the N-Controller, and without obstructions hindering the Bluetooth signal.

If after some time the N-Bath cannot be connected the following screen appears (Figure 4), with the message 'No Nterilizer devices found'. This is also confirmed by the message on the top right of the screen: 'Device Code: Not Available'. Check that the N-Bath is still powered on and, if necessary, move the controller closer to the device. Tap on 'SEARCH' to restart the search for devices.

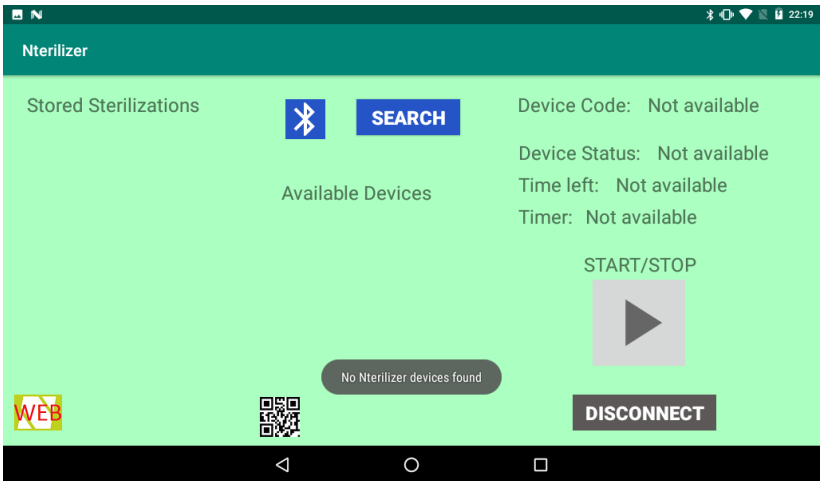

Figure 4. Search for devices failed

Note: The same error would appear also if the N-Controller is not associated with the N-Bath, a key step normally undertaken by the Nterilizer representative during the initial installation. If you experience some issues, please contact the Nterilizer representative or directly the manufacturer for assistance.

It is possible that N-Controller is able to find the N-Bath but not to automatically connect to it, as confirmed by the code of your device shown after 'Available Devices'. In this case it is sufficient to tap on the code of your device to initiate the connection (Figure 5).

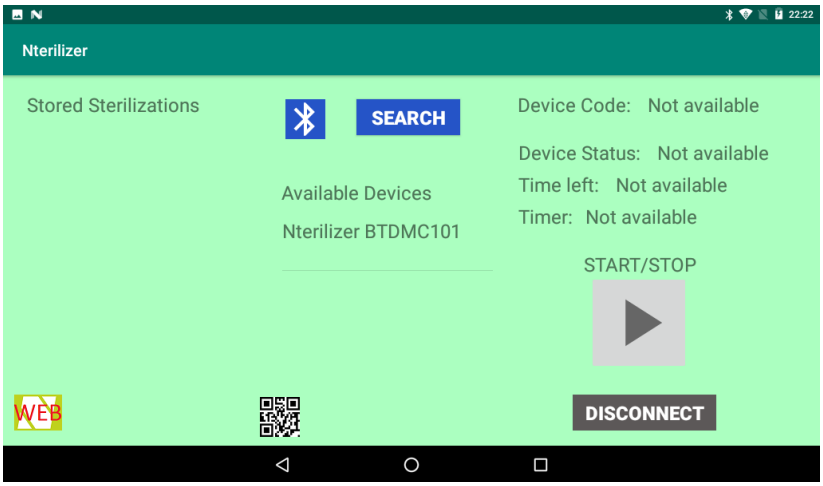

Figure 5. A N-Bath device has been found but is not connected

Once the connection is completed the message 'Device Code' will show the name of your device. The following screen appears (Figure 6).

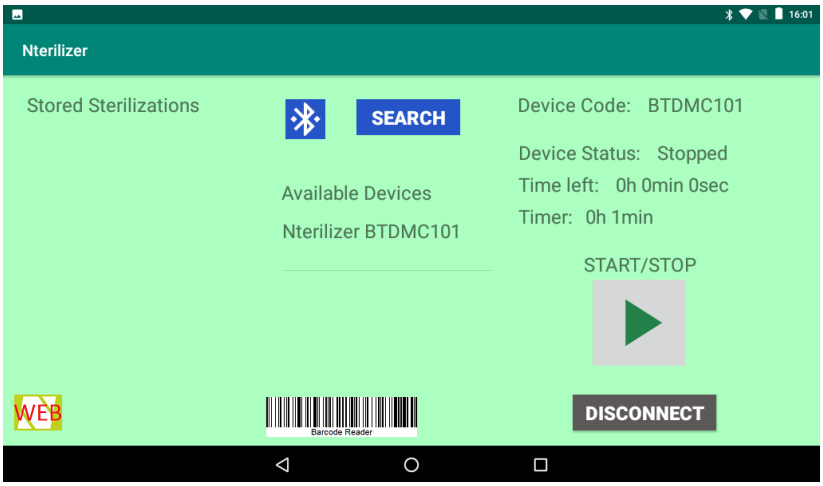

Figure 6. The N-Bath is connected

3. Start the sterilization

Check the connection status to ensure the device is still connected. Activate the N-Bath by tapping on ‘START/STOP’. The following screen appears (Figure 7).

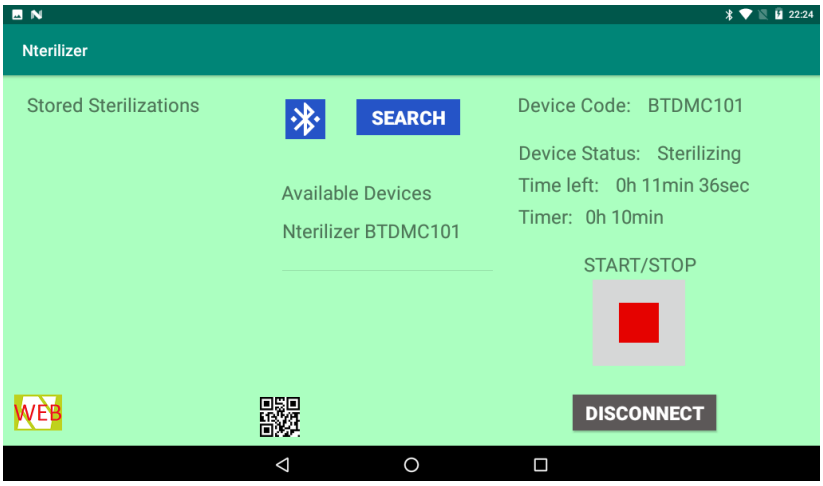

Figure 7. The sterilization process started

At the end of the sterilization process, which will last for approximatively 10 minutes, the field ‘Timer left’ will be showing ‘0h 0min 0 sec’.

If for whatever reason it is necessary to stop the process before its completion, it is sufficient to tap again on ‘START/STOP’ and the ongoing sterilization will be stopped.

4. Review sterilization data

At the end of the sterilization the message ‘Stored sterilizations’ shows the details of the completed process.

Tap on the sterilization number to open a window with the recorded data and review the information. The following screen appears (Figure 8).

If the sterilization process is not successfully completed, due to an error or because of a manual interruption initiated by the operator, the field ‘Energy’ in the details window will be showing ‘0’ and a reason for the process failure will be indicated.

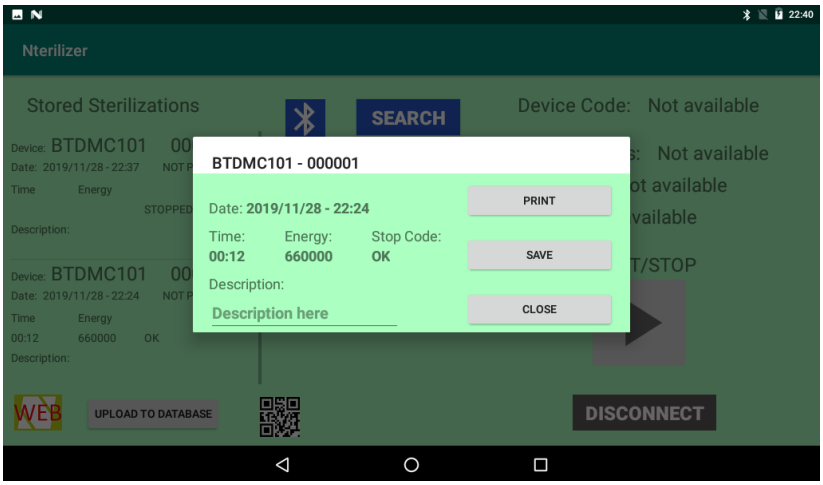

Figure 8. Window with details of completed sterilization

5. Add data, save and print

Tap on 'Description' to add required data, including the patient code (or the code of the carrier in case of Warming procedure) and additional notes.

Tap on 'Save' to save changes.

Tap on 'Print' to print the label with all recorded information about the completed sterilization. It is possible also to print the label in case the sterilization process is incomplete.

6. Ensure that the Wi-Fi network is on

Return to the home screen. The following view appears (Figure 9, same as on Figure 2). Tap on the Wi-Fi icon on the top right corner to open the network preferences window (Figure 10).

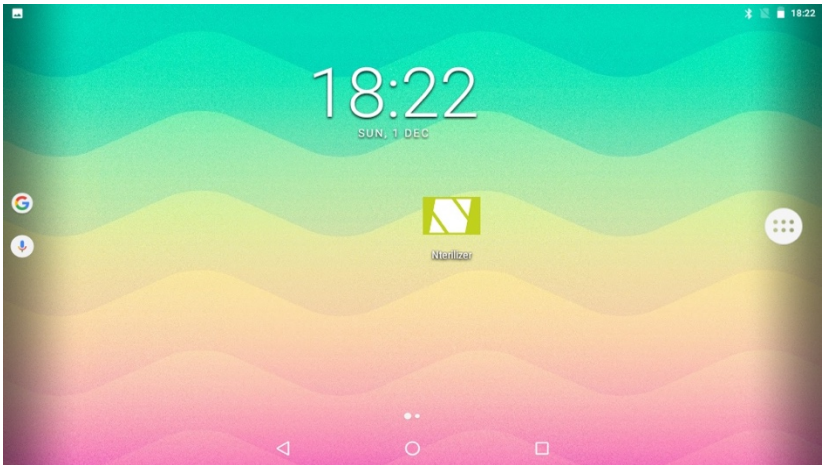

Figure 9. Return to the home screen

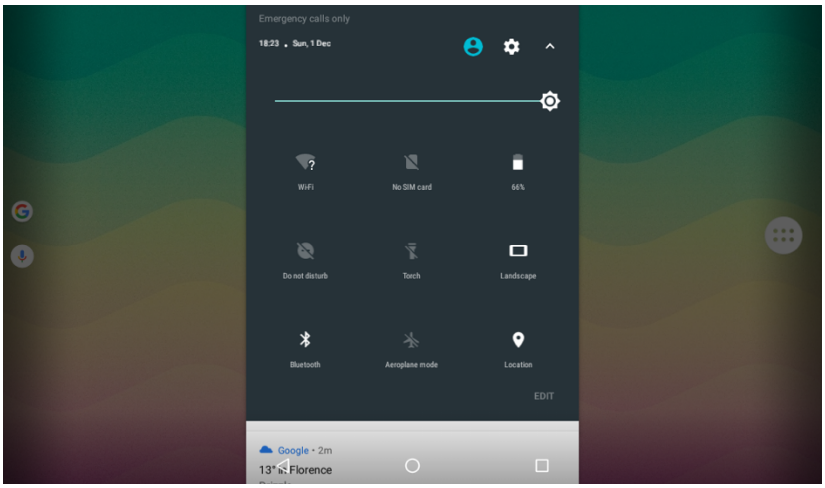

Figure 10. Open the network preferences window

Tap on the icon Wi-Fi to activate/disactivate the service. When Wi-Fi is active, a list with available networks appears (Figure 11).

Search for the network that corresponds to the provider for your laboratory and select it. When prompted for a password insert the access key for the Wi-Fi network selected (Figure 12). To facilitate entering long passwords, tap on the 'Show Password' which enables to view what is typed. When ready tap 'Connect' to activate the Wi-Fi network.

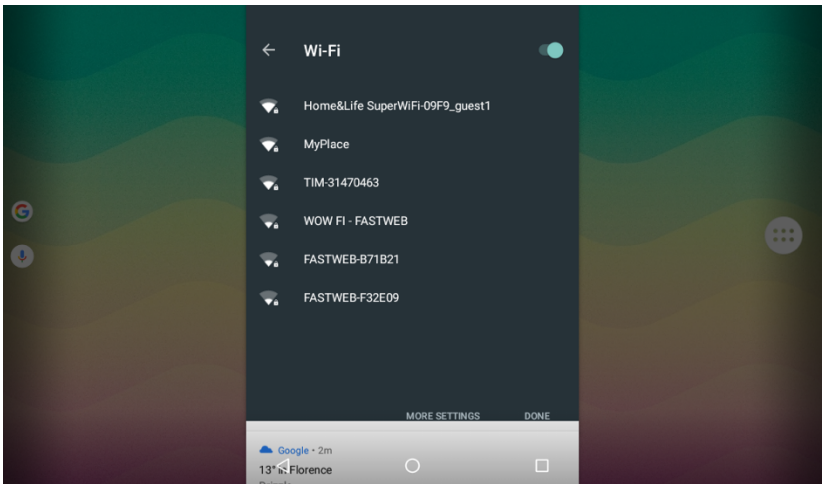

Figure 12. List of available Wi-Fi networks

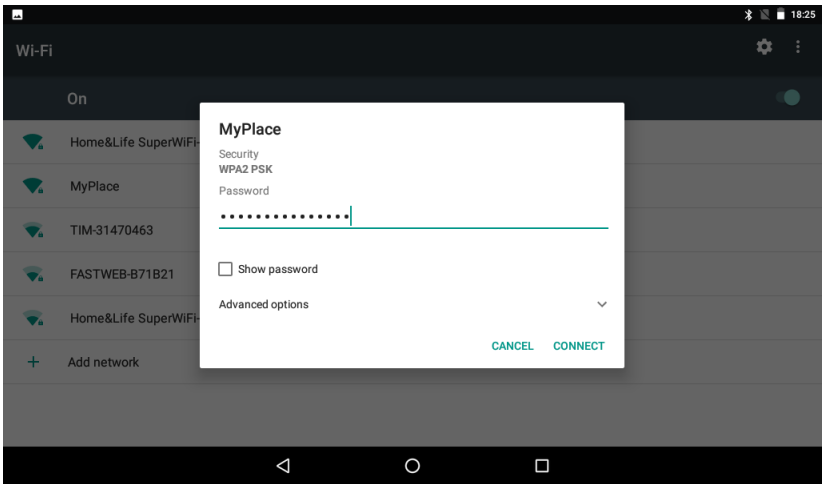

Figure 13. Enter password for selected Wi-Fi network

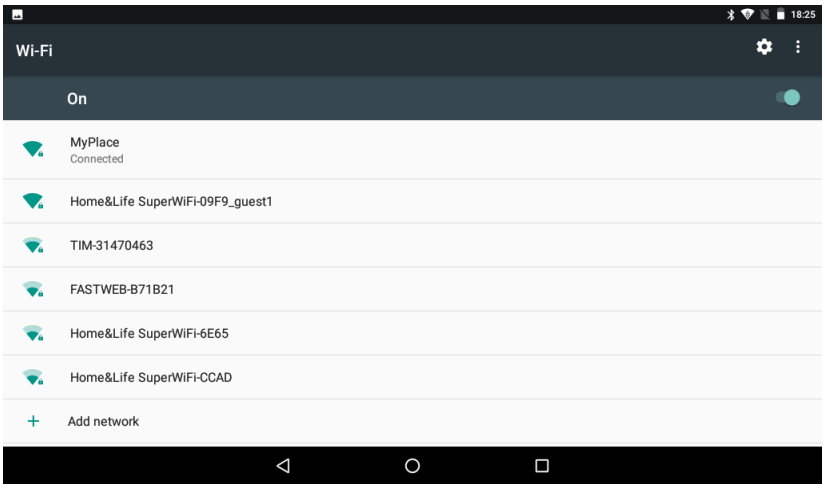

Figure 14. Wi-fi password correctly entered

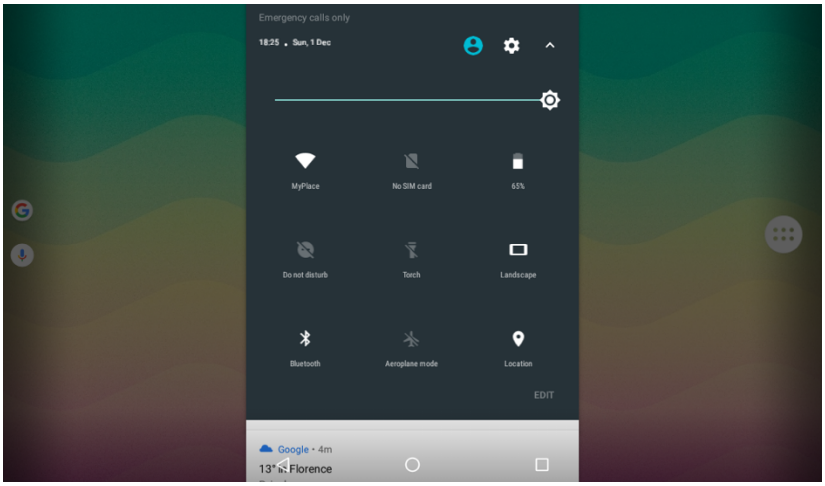

Figure 15. Selected Wi-Fi connected

7. Upload sterilization data

Upon successful completion of sterilization (refer to step 6 on previous section), tap on 'UPLOAD TO DATABASE' to save the sterilization data on the Nterilizer dedicated web portal.
Refer to the following section for more details on security of the Nterilizer portal.

Note: **The initial configuration of the devices and Nterilizer portal is normally undertaken by the Nterilizer representative during the set-up installation. For any difficulties, please contact the Nterilizer representative or directly the manufacturer.**

Part C. Nterilizer Web Portal

This section provides a description of the protection methods used by Nterilizer to guarantee the security of sterilization data.

Nterilizer uses a dedicated web portal, which is based on blockchain technology. The tablet sends data to the portal in the form of text. The portal then encrypts the data with an asymmetric key which utilizes RS256 as encryption code. The encrypting keys are specific to a single tablet, allowing each individual data sender to be identified. The system also ensures that the identity of the sender is certified before uploading data, providing extra security of the data.

Furthermore, the system used to send data from the tablet to Nterilizer server is a HTTPS protocol, therefore is protected by an SSL encryption layer. This makes the data illegible for outsiders intercepting it, unless the SSL is decrypted. Because this is technically feasible, the use of the unique key described above guarantees that data cannot be altered.

Essentially, the security of data transmitted via Nterilizer device is at least as secure as credit cards data sent to a secure website. Furthermore, Nterilizer is ensuring data will not be altered before arriving at the server.

Nterilizer recommends that users register patient data in the coded form which is used in most laboratories. In this way patient confidentiality is fully protected.

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APPENDIX II N-Test (UV lamps quality control) detailed procedure

1. Introduction

The N-Bath should be validated prior to first use, and periodically inspected and maintained in accordance with the manufacturer's instructions. This section describes how to utilize the N-Test to complete the periodic checks to test the effectiveness of the UV rays released by the UV lamps. The N-Test is based on a 2017 study published by SIERR (Italian Society of Embryology, Reproduction and Research). For more information visit the *Bibliography* section of Nterilizer Website).

Note: **The initial validation of the N-Bath is normally undertaken by the Nterilizer representative during the set-up installation. For any difficulties with undertaking subsequent N-Tests, please contact the Nterilizer representative or directly the manufacturer for assistance.**

2. Test frequency

The frequency of the periodic checks is to be assessed based on the number of hours of use of the N-Bath. The device is provided with an in-built system that counts the number of hours of sterilization delivered. The system informs the user when the hours of radiation reach the maximum recommended before each test. At this point to ensure effectiveness of subsequent sterilizations the user is asked to use the N-Test to check the condition of the UV lamps.

3. Test kit

The N-Test includes two culture plates (90mm standard petri dish) with a large number of spores of Geobacillus Stearothermophilus (ATCC 7953) in a suitable growing medium (Mueller Hinton agar). One of the two culture plates is to be used for undertaking the test inside the N-Bath, Test Plate, and the other is to be used as a control, Control Plate (Figure 1).
The kit includes also a removable label to be used to identify the control plates during the testing process.

Figure 1. Test plate placed inside the N-Bath and Control plate placed outside

4. Test initiation

Remove one culture plate from the package, take the removable label and write on it Test plate and the date/time of testing, attach the label on the culture plate. This is now the Test plate. Deposit the Test plate, without opening it, at the bottom of the N-Bath.

Start the sterilization cycle as usual (refer to Chapter 3). Approximatively 10 minutes of radiations will be delivered to the Test plate.

5. Culture plates incubation

Once the sterilization of the Test plate is successfully completed remove it from the N-Bath and place it inside the incubator.

Then remove the control plate from the pack and place it in the incubator next to the culture plate. Set the incubator at a temperature of 60°C and the timer to 4 hours.
Test the culture plates to assess the level of bacterial growth.

6. Test Results

Following sufficient incubation, the Test plate, which has been exposed to the UV radiation, is supposed to be 100% free of bacterial growth whereas the control plate, which has not been exposed to the UV radiation, is supposed to be loaded with bacteria.

If the Test plate still shows the presence of bacterial growth the UV lamps are to be replaced. Upon completion record the test results in the sheet enclosed with the kit and/or upload the data to Nterilizer web portal.