

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 and switch it on.	
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 <i>Available Devices</i>).	
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 approximately 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 together 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 Nsterilizer web platform.	
18	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.	
19	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 each time a new sterile N-Sleeve	
20	Repeat the procedure from Step 1 for each patient.	

ADDITIONAL 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.	