

Operating Instruction & Manual
For Clotho Drive Unit's
covers both the single and dual channel models



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Content

1. Concept overview	3
1.1 - Product purpose	3
2. - Device Overview	3
2.1 - Clotho internal design.....	5
2.2 - Requirement	5
2.3 - Specification.....	5
3. Software setup.....	6
3.1 – Basics is the human brain and heart	7
3.2 – You can program various parameters.....	7
3.3 – How to program Clotho	9
3.4 – How to start and stop the process software.....	10
3.5 – Details to how measurements is done.....	11
3.5 - Alarms	12
3.6 – Overview	12
4. Hardware installation.....	13
4.1 - Fault information	15
5. Communication.....	15
5.1 - PID routines.....	16
6. Operation Manual.....	16
6.1 - Operation principles	16
6.2 - Wi-Fi connection	17
6.3 – LAN IP/TCP connection.....	17
7. Safety precautions	17
7.1 - Documentation	17
7.2 – Definitions.....	17

1. Concept overview

Clotho is a family of Single-Use-Pump (SUP) Drive Units designed for operation of **CellMembra** or **CellRetention** Perfusion-Single-Use-Bioreactor. Integrating either the Clio One-way-SUP (O-SUP) or Thalia Alternating-SUP (A-SUP) diaphragm pump. Clotho is available in both mono / single channels and stereo / dual channels models.

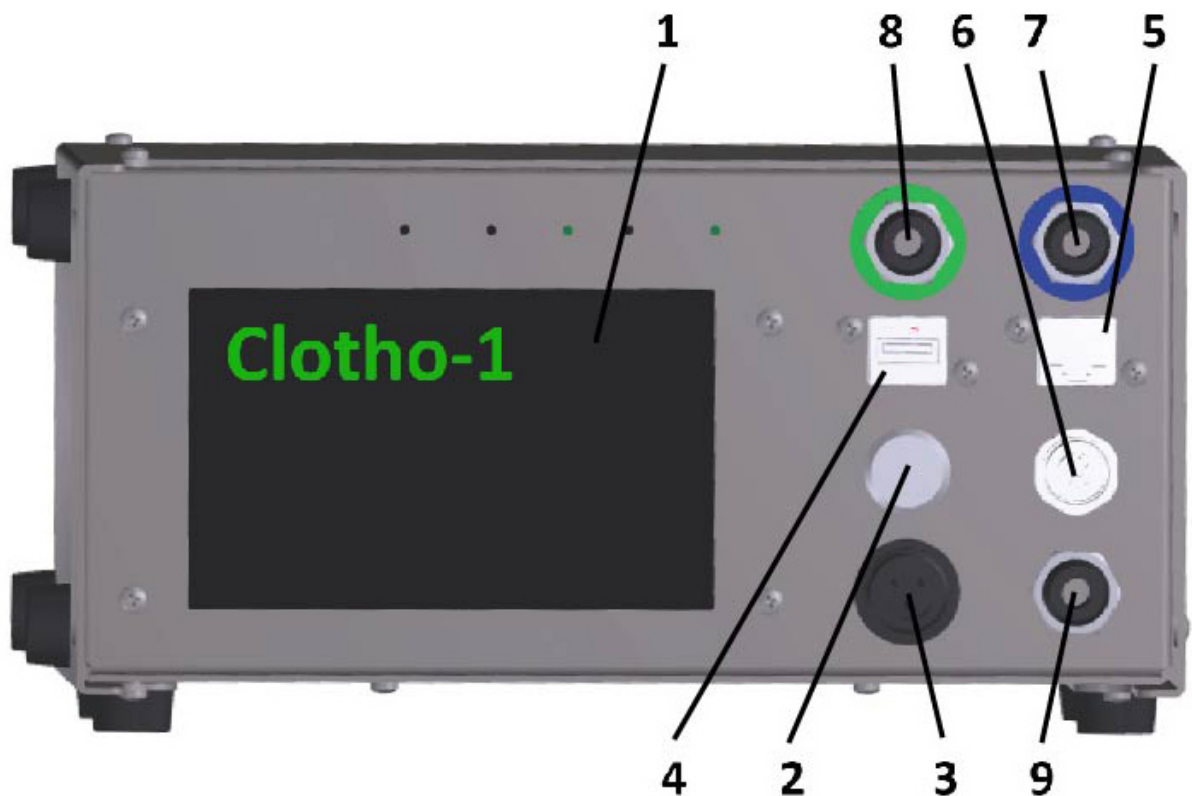
1.1 - Product purpose

www.perfusecell.com manufacturer the products CellMembra and CellRetention. Both products are Single-Use-Bioreactor's for mammalian high cell density cultivation operating in perfusion mode - Continuous Processing. Both SUBs integrate a diaphragm Single-Use-Pump. Either a O-SUP or A-SUP and one or more stacked hollow fibre module(s) / Cross-Flow-Filter (CFF) - all in one package. The process concept to choose from is either: PTF – Pulsating-Tangential-Flow (Clio O-SUP) or ATF – Alternating-Tangential-Flow (Thalia A-SUP).

Driving the diaphragm SUPs requires supply gas both below and above atmospheric pressure. The drive gas is then constantly adjusted via a set of proportional valves inside Clotho. Control is based on input from sensors (distance and pressure) given to the build-in CPU. Stroke Volume (SV) and number of strokes, Beats-per-Minute (BpM) determine the pumped broth volume, Cardiac Output (CO) and velocity (m/s) of the broth passing through the CFF.

2. - Device Overview

The Clotho Drive Unit is a product manufactured by sister company www.pumpcell.com for PerfuseCell. Clotho mono & stereo are both housed in the compact Hephaestus U2 cabinet manufactured from AISI 304 stainless steel.



For single channel, Clotho-1 front panel is equipped with:

1. 5" touch sensitive TFT display
2. Main power breaker
3. Buccaneer 24 VDC power input
4. USB socket for Wi-Fi access and charging
5. RJ45 socket for Local-Area-Network (LAN) connection
6. SUP laser sensor input with M12, RKF5, 5P socket
7. Supply pressure 6 mm one-touch connection
8. Supply vacuum 6 mm on-touch connection
9. SUP drive via 6 mm hose and on-touch connection



For dual channel, Clotho-2 front panel is equipped with:

1. 5" touch sensitive TFT display
2. Main power breaker
3. Buccaneer 24 VDC power input
4. USB socket for Wi-Fi access and charging
5. RJ45 socket for Local-Area-Network (LAN) connection
- 6L. Left channel laser sensor input with M12, RKF5, 5P socket
- 6R. Right channel laser sensor input with M12, RKF5, 5P socket
7. Supply pressure 6 mm one-touch connection
8. Supply vacuum 6 mm on-touch connection
- 9L. Left channel SUP drive via 6 mm hose and on-touch connection
- 9R. Right channel SUP drive via 6 mm hose and on-touch connection

There are two fan openings on the rear panel of Clotho. Avoid pushing the cabinet close to a wall prohibiting ventilation and internal cooling.

2.1 - Clotho internal design

Internal sensors and actuators:

- Pressure sensor for supply, >1 - <4 bar atmospheric (will tolerate up to 8 bar supply pressure)
- Vacuum sensor for supply, <0,1 bar absolute (will tolerate up to 8 bar supply pressure)
- First channel drive gas pressure / vacuum sensor outlet to SUP, ± 1 bar
- Second channel drive gas pressure / vacuum sensor outlet to SUP, ± 1 bar
- Proportional valve for positive drive gas pressure regulation
- Proportional valve for negative drive gas pressure regulation
- Fan for cabinet temperature control

External sensor(s):

- For each channel, a re-usable tri-angular red Leuze Laser sensor mounts on the re-usable bracket, foot on the SUP for on-line measurement of the actual position of the free-floating silicone membrane / diaphragm inside the Clio SUP and Thalia SUP.



Two identical red Leuze Laser sensor's shown with 1,5 meter wire and M12 connectors.

2.2 - Requirement

The Drive Unit must be properly installed according to fluid diagram and general recommendations. The drive gas inlet ports are marked. So, for vacuum a green disc suggest green 6 mm hose and a blue disc for pressurized air suggest using a blue 6 mm hose.

2.3 - Specification

When Clotho is connected to:

- a suitable supply of pressurized air with sufficient capacity - above 1,2 Bar
- a suitable supply of vacuum with sufficient capacity – lower than 900 mBar
- Leuze Laser sensor(s) for each either one or two channels
- Clio and/or Thalia size 30 or 100 Single-Use-Pump(s) for channel A or A + B
- 24 VDC supply

then the specification for both single and dual channels models is:

Beats-per-Minute (BpM), strokes per minute	Check latest info on www.perfusecell.com
Cardiac Output (CO)	Check latest info on www.perfusecell.com
Stroke volume (SV)	Check latest info on www.perfusecell.com
GUI	5" touch sensitive TFT display
Computer power	900 MHz quad-core ARM Cortex-A7 CPU
USB socket	Wi-Fi like NetGear N300 Mini Adaptor
RJ45 socket	IP/TCP via LAN (ModBus protocol)
Power supply	24 VDC, >30 watt via Buccaneer socket
Build in fan, noise level, dBa	<65
Duty cycle	100%
Orientation	any
Operating conditions	10°C to 40°C, <80% relative humidity, non condensing
Life time, estimated, hours	<50,000
MTBM, battery must be changes, hours	10,000
Cabinet size and material	Hephaestus U2 – AISI304
Drive Unit weight, kilo	4,5

3. Software setup

The screenshot displays the Clotho-2 GUI with the following elements:

- Top Bar:** Version C.68, navigation buttons for 'Go to channel B' and 'Go to overview', the title 'Clotho-2', and a digital clock showing 2017-12-20 11:05:59.
- Control Panels:**
 - Cleaning/Harvest relation:** 1 : 0
 - Set point pumped volume:** 0 ml/min
 - Velocity calc. straws & diameter:** 1 straw, 1.0 mm diameter
 - Configuration:** Close
 - Automatic pump control:** Run, Stop
 - Manual pump control:** Vacuumize, Pressurise
- Adjust Time Panel:** 'Wanted:' section with input fields for 0 Y, 0 M, 0 D, 0 h, 0 m, 0 s, and a 'Set Time' button.
- Pump selection Panel:** Dropdown menus for 'PTF:' and 'ATF:'.
- Data Table:**

Pump Pressure:	Realtime vol. (ml/min sec):	Pump stroke per min:	Last harvest avg. velocity:
-0.008 Bar	0.0 ml/min	0	0.0 m/s
Sup. Pressure:	Avg. vol. pumped:	Pump stroke before cleaning:	Last cleaning avg. velocity:
-0.010 Bar	0.0 ml/min	999	0.0 m/s
Vac. Pressure:	Total volume pumped:	Total pump stroke:	Total run time:
-0.011 Bar	0.0 ml	0	0.0 min
- Graph:** A line graph with a y-axis from 0 to 80 and an x-axis from 0 to 1m, showing a flat line at 0.

Graphical-User-Interphase (GUI) for Clotho Drive Unit operate with different colors. Light red is warning info from pressure sensors. Dark green lower left rectangles is Apollon PLC information. At right you choose the SUP principle – either PTF or ATF and the actual SUP size being 100% SV.

3.1 – Basics is the human brain and heart

Clotho control the cardiac cycle of the Clio of Thalia pump. The parameters we work with are:

- Cardiac Output (CO) = total volume pumped, SUP capacity, ml/min (CO = BpM x SV)
- Beats-per-Minute (BpM) = determined by the diaphragm diameter
- Stroke Volume (SV) = programmable from 10-100% in 10% section

Clotho Drive Unit copies the purpose of Medulla in the human brain and the heart's Sinoatrial Node and combines the features with the ability for interaction, programming, and functionality. Clotho take advantage of the build-in Apollon brain and receives real-time signals from pressure, temperature, and displacement / Laser sensor(s). Apollon regulate by positive feedback control a set of proportional valves for the BpM, and the driving force and movement of the elastic Myocardium wall inside the Clio and Thalia SUP.

3.2 – You can program various parameters

The internal clock is set from factory to CET, Central-European-Time. You should check if the clock is relevant for your geographic area. Clotho is equipped with a battery driving internal clock for accurate time measuring. If you need another time its easy to alter by pressing "Open" under "Configuration". Remember to "Set" the time after changes.



The window "Open" allow clock modification and SUP selection. If clock time is OK or you have modified and chosen the correct size SUP, you have finished - then press "Close".

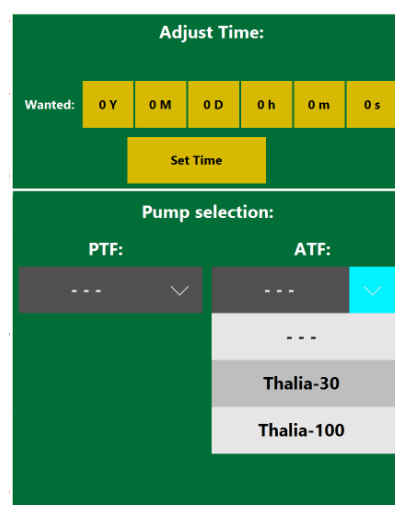
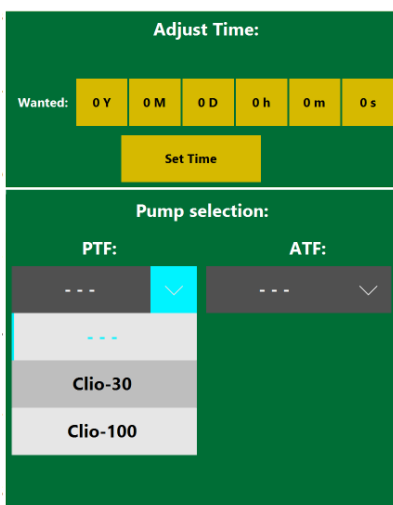
At start-up most often, no SUP size is chosen. Press Configuration "Open" and the window for clock adjustment and SUP selection pops up.



Adjusting time to alter the pre-set time.



The numpad will show when to choose the value of time.



If the black bar shows “Disabled” = no SUP is chosen. Press the “- - -” black bar and a scroll down window appear. Choose the correct size SUP and the black bar show the SUP chosen. Press then “Close” in the yellow “Configuration” window.

3.3 – How to program Clotho

Depending on the Drive Unit software version you can determine:

1. SP (Set Point) flow = total pumped volume in ml/min ($CO = BpM \times SV$)
2. Stroke Volume (SV) = programmable in step of 10% from 10 - 100%
3. Velocity Calculation m/sec = through the CFF ranging 1-99 straw and straw diameter
4. Ratio between Harvest cycles and Cleaning cycles.

There is a relation between SV and BpM (Beats-per-Minute). If you select a low SV the maximum BpM depending on the Cloio size determines the CO (Cardia Output). Maximum CO occurs with SV = 100%.

Ver. C.68	Go to channel A	Go to overview		Clotho
Cleaning/Harvest relation:	Set point pumped volume:	Velocity calc: straws & diameter		Configuration:
1 : 0	0 ml/min	1	1.0 mm	Close
Automatic pump control		Manual pump control		
Run	Stop	Vacuumize	Pressurise	

Programming and input window for channel A

Straw number and straw dimension must be selected. Straw range from 1 – 99 and straw diameter with 0,1 mm step.

We suggest looking at www.perfusecell.com/support/clotho-drive-unit where its explained detailed.

- Cardiac Output (CO) = total volume pumped, SUP capacity, ml/min ($CO = BpM \times SV$)
- Beats-per-Minute (BpM) = ranging 0 - 30 BpM, SUP diameter/stroke volume depending
- Stroke Volume (SV) = programmable from 10 - 100% of SUP specification

Ver. C.68	Go to channel A	Go to overview		
Cleaning/Harvest relation:	Set point pumped volume:	Velocity calc: straws & diameter		Configuration:
1 : 0	0 ml/min	1	1.0 mm	Close
Automatic pump control		Manual pump control		
Run	Stop	Vacuumize	Pressurise	

Programming and input window for selected channel B.
 Top line for programming is: 1:0 – 0 ml/min – 1 – 1.0 mm – Close



Numpad shown when selecting the value of the SP flow, cleaning/harvest or velocity calc.

3.4 – How to start and stop the process software

Automatic pump control		Manual pump control	
Run	Stop	Vacuumize	Pressurise

Run

Light green (channel A) / Yellow (channel B) button - start up the process based on latest program.
Light green and Yellow will shift, flip depending on which channel is selected for view.

Stop

Light green (channel A) / Yellow (channel B) button - terminates the process based on latest program.

Vacuumize

Light green (channel B)/ Yellow (channel A) button for manual operation. Use for system priming and testing functionality.

Pressurize

Light green (channel B)/ Yellow (channel A) button for manual operation. Use for system priming and testing functionality.

3.5 – Details to how measurements are done

Pumped volume, Cardiac Output (CO mL per minute)

SUP displacement and stroke frequency determines the total volume over time. The SUP will pump double the programmed volume as the pump stroke is 50% of the time. The display shows programmed curve and the actual curves. The pumped volume, CO cannot exceed the SUP specific capacity multiplied with the SV in percent.

Stroke Volume, SV, 10 – 100 % (late software versions)

Potentially shear stresses could be of interest. Find the smallest part in the fluid pass and measure the diameter to be inserted in red window under “Velocity Calculation.

CFF velocity, m/s: 0,1 – 10

Show actual, on-line velocities in meters per second as an average based on the number of straws you have programmed into the software.

Repetitions

The user programming for Harvest / Cleaning ratio. Could be only Harvest cycles. Could be one Cleaning cycle for each 10 Harvest cycles. A Harvest cycle will seek to obtain the fastest possible single stroke for highest possible Cleaning velocity through the straw.

Pump Pressure:	Realtime vol. (ml/min sec):	Pump stroke per min:	Last harvest avg. velocity:
-0.009 Bar	0.0 ml/min	0	0.0 m/s
Sup. Pressure:	Avg. vol. pumped:	Pump stroke before cleaning:	Last cleaning avg. velocity:
-0.009 Bar	0.0 ml/min	999	0.0 m/s
Vac. Pressure:	Total volume pumped:	Total pump stroke:	Total run time:
-0.011 Bar	0.0 ml	0	0.0 min

Data acquisition and real-time read out windows.

Its good to know that “Real-time flow” is approximately the double of “Average-flow” of the Clio R-SUP. This because the SUP is a reciprocating pump and fluid is moved only at every second stroke. Different for the Thalia A-SUP which do not really pump but move a SV forth and back.

“Total volume moved” can only be from process start after pressing Run button.

3.5 - Alarms

Data from drive gas is shown online. So it the actual drive gas pressure on the gas side of the Myocardium diaphragm jumping between vacuum and pressure.

Alarms for:

Alarm area will show if the supply hose for pressure and vacuum is not mounted correctly.

- Pump pressure range, Bar: ± 0.5 to 0.9
- Supply pressure range, Bar: 0.5 to 1.1
- Vacuum pressure range, Bar: ± 0.8 to ± 1.0

If no pressure or vacuum is available, drops out or insufficient – then an alarm appears as red and the process stops.

3.6 – Overview

Overview is the screen where it is possible to have the total control view for channel A and channel B simultaneously. The SUP action graph and values are shown for both.



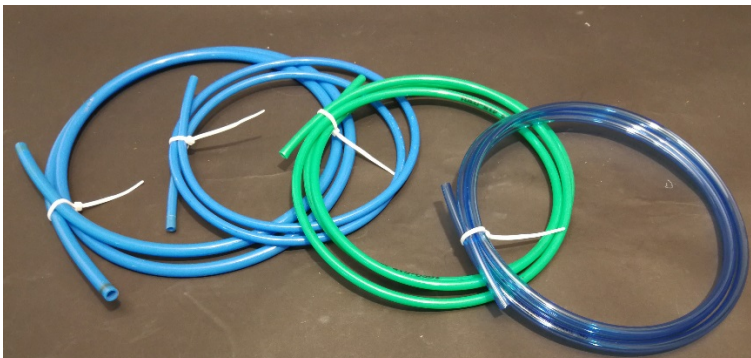
The Clotho overview screen with both channels on the Clotho-2 model.

4. Hardware installation

The scope of the Clotho product is to drive the CellMembra integrating the Clio O-SUP or CellRetention integrating the Thalia A-SUP. Unpack and dispose in a nice way or store the shipping material properly. Check out that you have all the parts shown here.

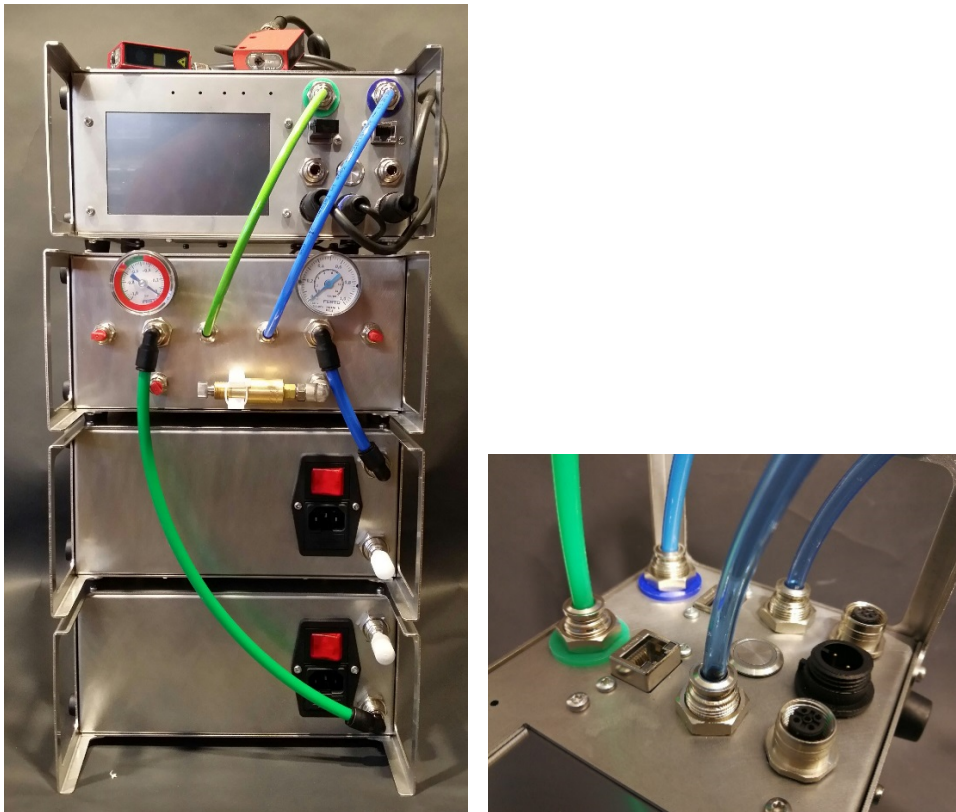
1. Clotho in U2 cabinet
2. Laser sensor(s) – red little box with black cable
3. Power Supply model Zephyros – one per Clotho – both mono and stereo models
4. NO power input cable to Zephyros supplied – you have tons of these standard cables anyway and with the correct wall plug for your application
5. Transparent blue soft 2 meter long polyurethane hose for drive gas connection

Depending on how much gear you received there will be or not included hoses in different colours.



We recommend using these colours and follow our recommendations. Light blue 6 mm nylon hose for pressurized air. Green 6 or 8 or 10 nylon hoses for vacuum depending on Clotho size. Transparent dark blue very flexible polyurethane for drive gas connection from Clotho to SUP.

Focus with these photos is how SUP and Power Supply (PS) is connected. PS connection is simple with the round bright blue finger nut on the black Buccaneer cable connector. Drive gas to the SUP is via the transparent blue soft polyurethane hose. Laser sensor input via the black M12 cable connector.



Be sure you connect vacuum to the left / green one-tough fitting and pressurizes air to the right / blue one-tough fitting. Find more information on how to take advantage of the Sarpedon unit here – www.perfusecell.com/download/drive-unit-manuals

Procedure to follow for model Clotho-1 and Clotho-2:

1. Connect a pressurised drive gas supply of max 1 Bar (could be Sarpedon / Alagonia) with 6 mm rigid blue hose.
2. Connect a vacuum source (such as Sarpedon / Alagonia) with 6 mm rigid green hose.
3. Connect the 6 mm soft transparent blue drive gas hose from the SUP to Clotho.
4. Mount the red Laser sensor on/in the support foot.
5. Connect 24 VDC from one of the Eos family of power supplies.
6. Start-up Clotho and watch the unit get alive
7. Spend some minutes and use the two manual buttons to remove air and fill SUP with liquid.
8. Program according to you SOP and planning
9. The Clotho is ready for action

Requirement

The system must be properly installed and connected in accordance with the specifications and previous information. Operator must also have gained familiarity with the Safety Instructions to be found separately on www.perfusecell.com/support/safety-instructions

Clock

Clotho unit need no up-front clock programming and is ready for installation. The Drive Unit is pre-programmed with the CET time. If you need different time look under section 3.2

4.1 - Fault information

The build-in display of Clotho will inform about possible faults whenever detected.

- If no air pressure or vacuum source is detected
- If no movement is detected by level sensor

5. Communication

Clotho contain a webserver displaying online information on the build-in display.

Each Clotho product has an IP address shown at the Manufacturer’s Identification Label under the product. If you hook up via the Wi-Fi connection to a touch sensitive PC, smartphone, or PAD you should see the GUI from the actual Clotho Drive Unit in a browser.



Illustration of the GUI shown on the build-in 5" display with channel A selected.



Illustration of the GUI shown on the build-in 5" display with channel B selected.

5.1 - PID routines

Many hours have been invested in setting up the PID routines and the intelligence for continues auto-tuning. Clotho will initiate auto-tuning at each start-up for best possible accuracy.

6. Operation Manual

At activation of power button then Clotho (or restoration of voltage after a power outage) starts up and the display will show:

- Firmware / operating system software (Linux) is loaded
- System configuration is loaded from memory
- Operating software (Clotho) is loaded and actual information is displayed

Any user-defined parameters from a previous process is stored in a battery-buffered memory and can be used for the next process. Clotho is pre-programmed from the PerfuseCell with simple routines. If there are no faults found by the software Clotho is ready to operate.

6.1 - Operation principles

Assuming a correct assembly of all systems, connections, etc. according to your Fluid Diagram and this manual – check all connections are tight.

6.2 - Wi-Fi connection

When Clotho is equipped with the included NetGear Wi-Fi access point the Apache webhost will be accessible from a browser. Go into “Settings”/ Wi-FI NETWORKS” on your PAD or smartphone and check if you can see Clotho and select. Return to a browser and write “anything” in the address line for access to Clotho.



Netgear mini Wi-Fi USB adaptor.

Software upgrade - <http://perfusecell.com/support/communication/software-upgrade/>

6.3 – LAN IP/TCP connection

For programming purposes and communication with PCS.

7. Safety precautions

Various component requires individual attention. Operator must also have gained familiarity with the Safety Instructions to be found separately on – www.perfusecell.com/support/safety-instructions

7.1 - Documentation

Clotho functionality must be checked on a regular basis and data of such testing kept recorded. As found here – www.perfusecell.com/support/documentation

Pumping capacities found here

<http://perfusecell.com/support/cellmembra-p-sub-series-30/clio-single-use-pump/>

<http://perfusecell.com/support/cellretention-p-sub-series-31/thalia-single-use-exchanger/>

7.2 – Definitions

Find here a glossary – www.perfusecell.com/support/glossary