



Laminar Wash™ HT2000 Station 96 User Manual

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Laminar Wash HT2000 User Manual

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Chapter 1:

General Information

Chapter Overview

- General Information
- Introduction to the Laminar Wash HT2000
- Chemical Compatibility
- Safety
- CE Mark
- Customer Service and Technical Support

General Information

In general, this user manual has been written for the purpose of providing technical, installation, operating and troubleshooting information to the operators of the Laminar Wash™ HT2000 Station. The content of this manual includes:

- How to set up and operate the HT2000
- The principle of operation and modes of function for the HT2000
- Safety features of the HT2000 and precautions to ensure safe operation
- Troubleshooting procedures and maintenance

Introduction to the Laminar Wash HT2000

The Laminar Wash solution enables sample preparation for multi-color flow cytometry, single-cell sequencing, and other cellular analytical methods. It bypasses the need of centrifugation for washing cells in suspension. The Laminar Wash technology is designed to maintain samples in a 96-droplet based format instead of using a conventional microwell with walls. It is a centrifuge-less processing method using the unique wall-less features of the Laminar Wash technology combined with a fully automated washer. The Laminar Wash HT2000 generates a laminar flow for each drop on the Laminar Wash 96-well Plate via dual-nozzle action, with one nozzle dispensing liquid into the drop and the other nozzle aspirating liquid. The Laminar Wash workflow is superior over the traditional centrifugation method.

Technical Specifications

Description	Specification
Physical	
Dimensions	310 mm H x 262 mm W x 302 mm D
Weight	14 kg
Electrical	
Voltage Requirement	100 - 240 V
Environmental	
Operating Temperature Range	4 - 28 °C
Operating Humidity	< 80%, non-condensing
Operation	
Plate Type	Laminar Wash Plate in 96-well format
Capacity	1 plate per operation
Performance	
Flow rate at nozzle	5-20µl/s
Volume capacity	80µl per nozzle
Wash sequence	96 wells simultaneous washing
Dilution factor per cycle	Approx. 3.5 times
Bulk flow rate for priming	Approx. 300ml/ min
User Interface	
Display	A single plus a double-digit display, 4 status indicating LED
Input control	4 input buttons
Number of washes per cycle	1x to 9x selectable
External Interface	
1x inlet	A connector for incoming fresh wash buffer
1x outlet	A connector for outgoing waste wash buffer
1x RS232	A plug-in for updating operating parameters

Chemical Compatibility

The components exposed to fluids and reagents are composed of materials which were selected to be resistant against common chemical substances. However, some common disinfectants should not be used when decontaminating the HT2000. Table 1-1 on page 4 lists the material composition of the main components of the HT2000, and shows some reagents which do not pose issues when in contact with these components. However, some reagents are not compatible with the component materials, and prolonged contact should be avoided to prevent corrosion and damage.

Component	Material	Approved Chemicals	Incompatible Chemicals
Internal base structure	Stainless Steel (304)	Ethanol, Benzene, Chloroform, Acetaldehyde, Propylene Glycol, Isopropanol, Formaldehyde, Phenol, Grease, Potassium Permanganate, Hydrogen Peroxide	Hypochlorite bleach, Sulfuric Acid
Some minor components of main body	Delrin (Polyoxymethylene)	Ethanol, Benzene, Soap Solutions, THF, Formaldehyde, Propylene Glycol, Isopropanol, Potassium Permanganate	Acetic acid, Grease, Ketones, Ozone, Phenol, Ammonia, Hypochlorite bleach, Iodine, Hydrogen Peroxide, Phosphoric Acid, Sodium Hydroxide (>50%)
Internal structural parts	Aluminium	Ethanol, Benzene, Propylene Glycol, Isopropanol, Formaldehyde, Ozone, Grease, Phenol, Hydrogen Peroxide	Hypochlorite Bleach, Soap Solutions, Sulfuric Acid, Potassium Permanganate, Phosphoric Acid
External structure			

Table 1-1: Chemical compatibility between the component materials in the HT2000, including common reagents and disinfectants. (Adapted from Ingersoll Rand Industrial Technologies, 2008 & K-mac Plastics).

Safety

User Attention Notifications

Several user attention phrases are used throughout this manual. Each phrase should draw the following level of attention from the user:

NOTE	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
CAUTION	Cautions users regarding potentially hazardous situations in regard to user injury or damage to the instrument if the information is not heeded.
!WARNING!	Warn users that serious physical injury can result if warning precautions are not heeded.

Chemical Hazards

!WARNING! CHEMICAL HAZARD

Some chemicals used can be potentially hazardous, and can cause injury or illness.

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing). For additional safety guidelines consult the MSDS.
- Do not leave chemical containers open.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazards

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Minimize contact with chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing).
- Use precaution when emptying the waste bottle.
- Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Material Safety Data Sheets



Some chemicals used with the HT2000 may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

MSDSs provide users with safety information needed to store, handle, transport and dispose of the chemicals safely. Curiox recommends updating laboratory MSDS records periodically.

Material Safety Data Sheets for Curiox reagents are available upon request by calling 650 226-8420 in the US or +65 6507 0361 outside the US. Otherwise call the chemical manufacturer directly or visit their web site.

Instrument Safety Labels

The following safety label is located on the HT2000. The label displays a safety alert symbol indicating a potential safety hazard.

Symbol	Description
	Pinch point hazard. Keep hands clear.
	Separate collection for electrical and electronic equipment.

Instrument Safety Features

Plate Detection

The HT2000 detects the presence of a Laminar Wash 96-well Plate. The machine will not begin operation if a plate is not detected on the feeder platform.

Power Supply

The universal power adapter is able to accommodate electrical power in the range of 100 - 240 V. The resultant output to the HT2000 power jack is 24 V DC.

Emergency Stop

Activating the emergency stop button in the middle of a washing cycle will cause the machine to immediately freeze. The machine goes to home position when the emergency stop button is subsequently deactivated.

Machine Homing

In the event power to the machine is cut off in the middle of a washing cycle and subsequently switched on again, the machine will go to home position.

Spill Tray

The spill tray sensor detects excess liquid in the tray to prevent it from spilling onto the machine's interior. When this happens, the error code "E31" is prompted on the machine display panel. User may only access the draining button during this time.

Safety Precautions

- At the end of a wash process, remove the plate from the feeder platform immediately to prevent the wells from drying, stressing cells and/or deteriorating biological integrity.
- Keep the area around the power supply free from liquid.
- During active operation, keep your hands off the instrument other than using the display panel.
- If there is an unexpected error, reset the instrument by turning the power switch off and back on.

General Precautions

- Do not use any other plate except the specified Laminar Wash 96-well Plate with the HT2000.
- Do not load more than one Laminar Wash 96-well Plate in the HT2000 at a time.
- Use only the supplied power adapter cord for electrical supply to the unit.
- Do not allow particles larger than 200 µm to enter any of the liquid tubes.
- Be careful not to spill liquid onto the interior of the HT2000.
- Always perform a cleaning cycle with an appropriate cleaning solution at the end of an experiment.
- Keep the original packaging material in case the unit should ever need to be shipped.
- Do not attempt to open or remove the instrument casing or motor parts. Doing so will void the calibration and warranty and may cause permanent damage to the instrument.
- Contact only qualified Curiox personnel for servicing of the HT2000.

Prior to System Operation

Ensure that all users of the HT2000 have:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Received instruction on handling of biohazards if biohazardous materials are to be used on the system.
- Read and understood all related MSDSs.

CAUTION

Avoid using the HT2000 in a manner not specified by Curiox. While the system has been designed to protect the user, this protection may be impaired if the instrument is used improperly.

CE Mark

Based on the testing described below and information contained herein, this instrument bears the CE mark.

Directive 2014/30/EU Electromagnetic Compatibility

This device has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1 for Emissions and Immunity.

Verification of compliance was conducted to the limits and methods of the following:

1. EN 61326-1: 2013 Emissions
 - a. Harmonics Current Emission (Class A)
 - b. Voltage Fluctuation/ Flicker
 - c. Conducted Emission (Group 1 Class B)
 - d. Radiated Emission (Electric Field) (Group 1 Class B)
2. EN 61326-1: 2013 Immunity
 - a. Electrostatic Discharge Immunity
 - b. RF Radiated Immunity
 - c. Electrical Fast Transient/ Burst Immunity
 - d. Voltage Surge Immunity
 - e. Conducted Disturbance Immunity
 - f. Voltage Dips & Interruptions Immunity

Directive 2014/35/EU Low –Voltage Device

This device has been verified and found to meet the requirements of Directive 2014/35/EU “electrical electronic equipment designed for use within certain voltage limits”.

Directive 2011/65/EU Restriction On the use of Hazardous Substances (ROHS 2)

This device has been verified and found to meet the requirements of Directive 2011/65/EU “restriction on the use of certain hazardous substances in electrical and electronic equipment”.

Directive 2012/19/EU Waste Electrical and Electronic Equipment (WEEE)

Dispose of the device according to Directive 2012/19/EU, on “waste electrical and electronic equipment (WEEE)” or local ordinances.

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Chapter 2:

Functional Description

Chapter Overview

- Introduction
- Functional Description

Introduction

This chapter gives a detailed overview of the parts of the HT2000 and the phases which outline a typical washing process.

Functional Description

The simple digital interface located on the top front panel of the HT2000 allows operators to adjust the number of desired wash cycles and the initial sample volume which will be shown numerically on the display. The main components of the HT2000 are shown in Figure 2-1, Figure 2-2 and Figure 2-3.



Figure 2-1: Front view of the Laminar Wash HT2000



Figure 2-2: Lateral (right side) view of the Laminar Wash HT2000

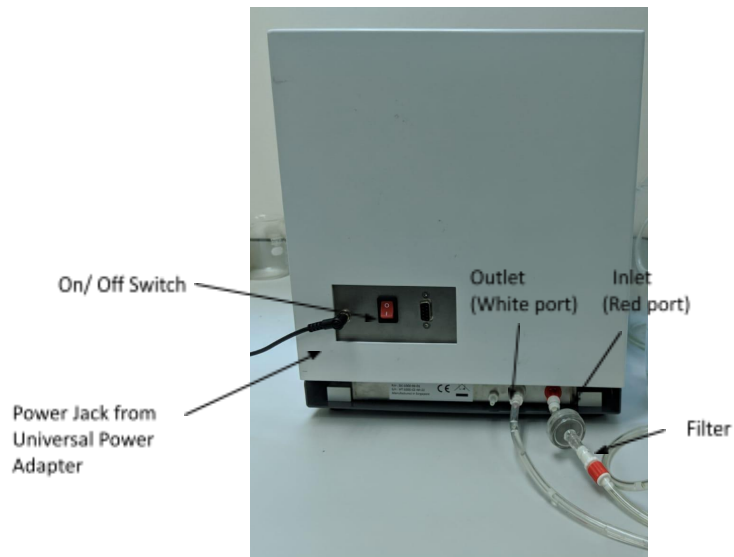


Figure 2-3: Back view of the Laminar Wash HT2000 with main components labelled.

Wash Cycle Process

The plate wash cycle process is as follows, a schematic representation of the process is shown in Figure 2-4.

1. Feeder platform detects for presence of plate and retracts into the machine.
2. Fluidics head lowers down into plate.
3. Based on indicated initial volume, dispensing head tops up or removes each well content until 80 μ l (e.g. if initial volume is 50 μ l, dispensing head adds 30 μ l to the droplet).
4. Aspirating head aspirates full volume based on internal firmware. It is intended that contact between aspirating nozzles and well droplet breaks before aspiration is completed. Once the aspiration nozzle completes the aspiration of existing drops at a given height of a nozzle, it continues to aspirate air. Resultant residual volume should be approximately 25 μ l based on the height of an aspiration nozzle.
5. Dispensing head tops up well contents based on internal firmware.
6. Steps 4 and 5 are repeated until stipulated number of washes is reached. Note: if only 1 wash is specified, the machine will only run until step 4.
7. Feeder platform extends out of the machine, returning plates with approximately 25 μ l residual volume in each well.



2 nozzles per droplet on Laminar Wash Plate. Cell washing via laminar flow in 2 to 4 mins.

Figure 2-4: Laminar Wash 96-well Plate washing process on the HT2000

Chapter 3:

Setup

Chapter Overview

- Installation
- Preparation for Operation

Installation

Upon arrival of the HT2000 package, check that all the accessories listed below are present. Some of the components of the HT2000 have been secured with brackets to protect against damage during transportation. Remove all brackets shown in the steps below before installing and powering up the HT2000.

List of items:

- Laminar Wash HT2000 Station
- Fluidic Head with Spacer
- Power Adapter (Output 24V, 3A)
- Electrical Power Cord
- RS232 to USB Cable
- Allen Key Set
- User Manual in USB Thumb Drive
- Declaration of Conformity (for CE compliant)
- Filter
- Plunger and Nozzle tool
- Spare 3.5 ID O-rings (10x Red, 3x Black)
- 1m Tubing, Red Coded Ring on Connector connected to Filter
- 1m Tubing, White Coded Ring on Connector
- Dummy Plate
- Calibration Plate
- 0.1m Tubing, Bare
- Buffer Inlet Bottle Cap
- 10ml Manual Priming Syringe
- Plunger and Nozzle tool
- Spare Nozzles & Plungers Set (Aspirating & Dispensing)

Installation

1. Place the HT2000 on a flat surface and in an environment with temperature and humidity conditions as stipulated in “Technical Specifications” on page 3.
2. Lift machine top cover and push hinge to lock the cover in the open position.



Figure 3-1: Lift the machine top cover (left) and lock in the open position (right).

3. Hold and pull down the two ends of the supporting bracket to remove. Please note that some force may be required for this step. The supporting bracket must be kept in a secured location for any transportation which may arise in the future. For convenience, we would suggest taping it to the back of the machine.



Figure 3-2: Remove the machine supporting bracket.

4. Using a size 3 Allen key, loosen bolts (x3) by turning to the anti-clockwise direction. Do not over loosen bolts. Move the locking bracket to the right.

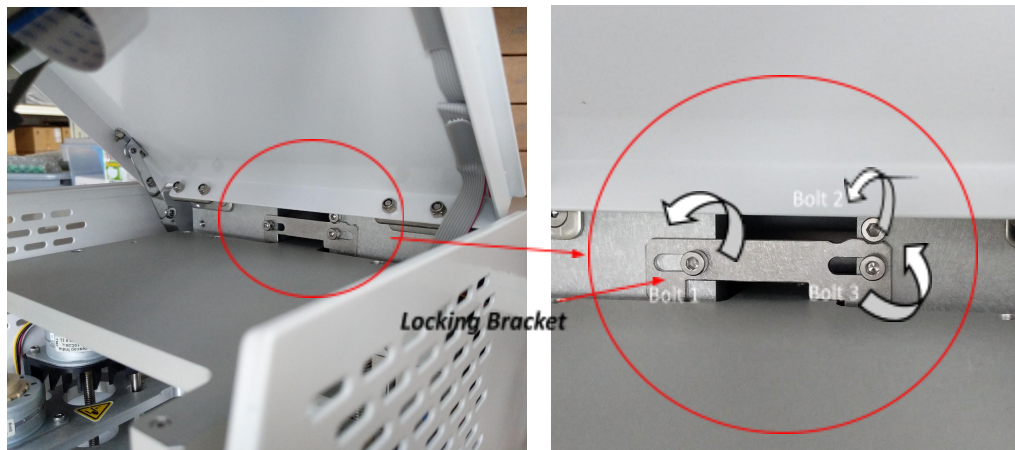


Figure 3-3: Loosen bolts to move locking bracket to the right.

- Once the locking bracket is on the right, use the same Allen key to tighten bolts (x3) and lock the locking bracket into position by turning to the clockwise direction. Do not over tighten bolts.

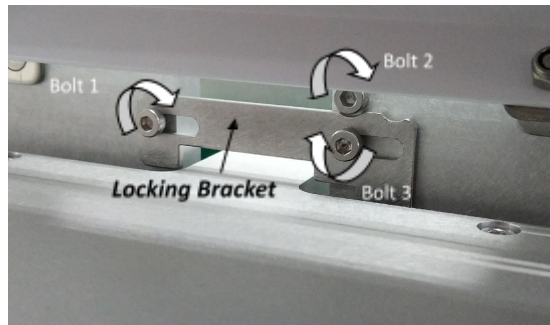


Figure 3-4: Lock locking bracket into position by tightening bolts

- Detach the cotter pins and lock pins, and then flip open the metal bars.

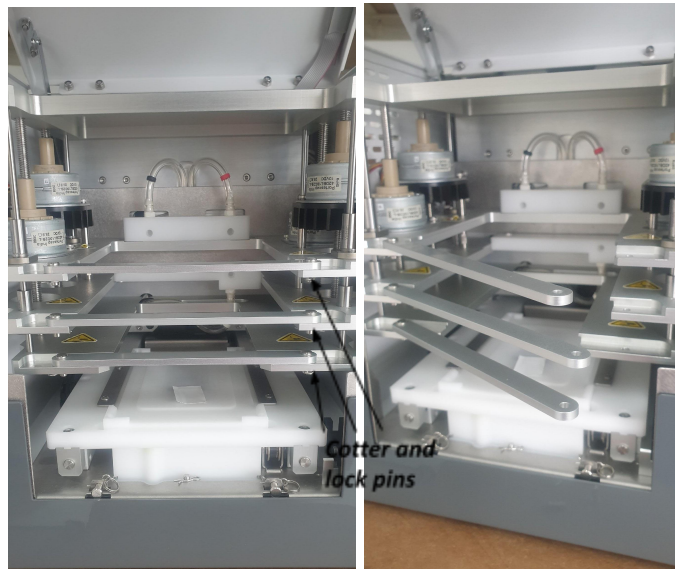


Figure 3-5: Opening metal bars

- Remove the fluidic head from its packaging, and wedge in the Fluidic Head Spacer from the accessories.



Figure 3-6a: Fluidic Head

And this is how it looks like underneath the nozzle block. Notice the fork on the right-side cuts in between the plungers when inserting

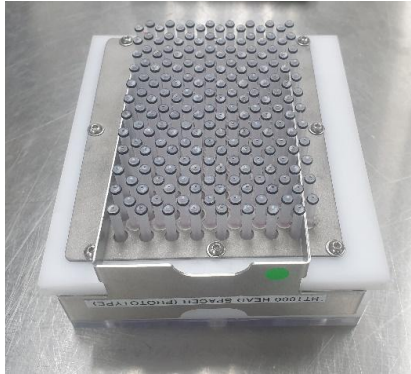


Figure 3-6b: Fluidic Head

8. Flip and align the centre block of the fluidic head to the machine.

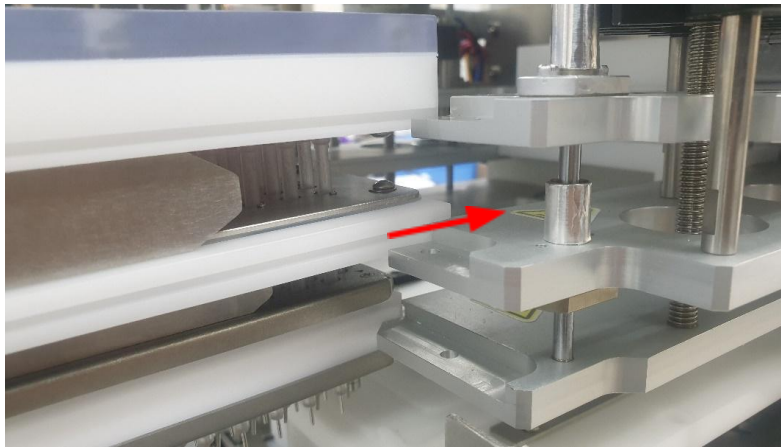


Figure 3.7: Align Fluidic Head to Machine (step 1)

9. Followed by the bottom block.

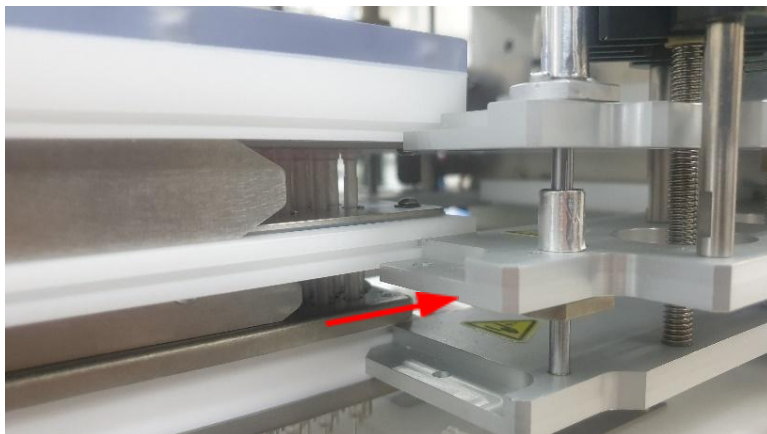


Figure 3.8: *Align Fluidic Head to Machine (step 2)*

10. And finally, the top block.



Figure 3.9: Align Fluidic Head to Machine (step 3)

11. Push the fluidic head all the way in before sliding the metal spacer out.

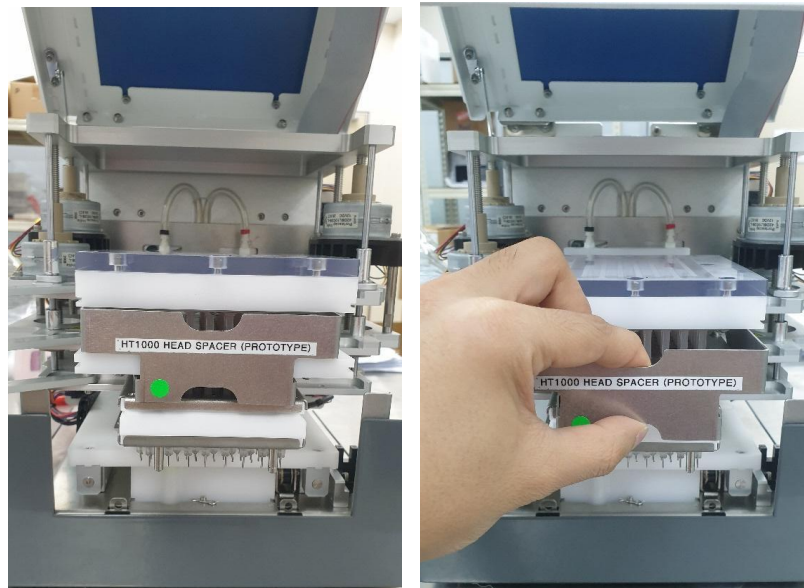


Figure 3.10: Align Fluidic Head to Machine (step 4)

12. Ensure the fluidic head is secured into position before closing the brackets.

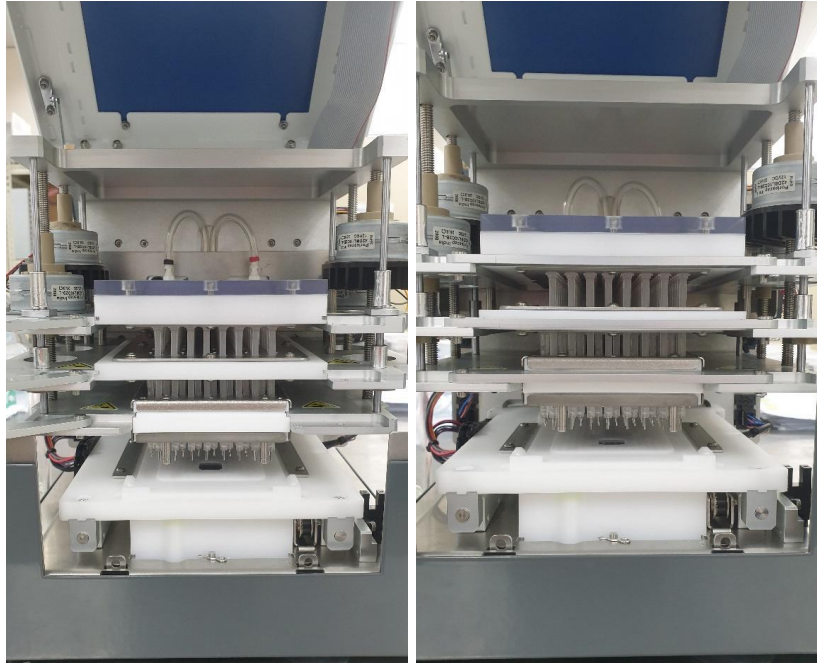


Figure 3.11: Align Fluidic Head to Machine (step 5)

13. Close the machine cover by returning the hinge back to its original position.

14. Connect the power cable to the universal power adaptor. Insert the power jack at the back of the HT2000 as shown in Figure 2-3.
15. Insert the two delivery tubes into the respective ports on the HT2000 in the manner specified below (Figure 3-1):
 - Install a clean filter into one end of the inlet tubing and insert into the red port on the HT2000. Insert the other end into wash buffer bottle via the buffer inlet bottle cap. Refer to the next section “Buffer Inlet Bottle Cap Installation” for installation instructions.
 - Insert one end of the outlet tubing into the white port on the HT2000 and the other end into the waste bottle.

NOTE: The buffer inlet bottle cap must not be used on the waste bottle.

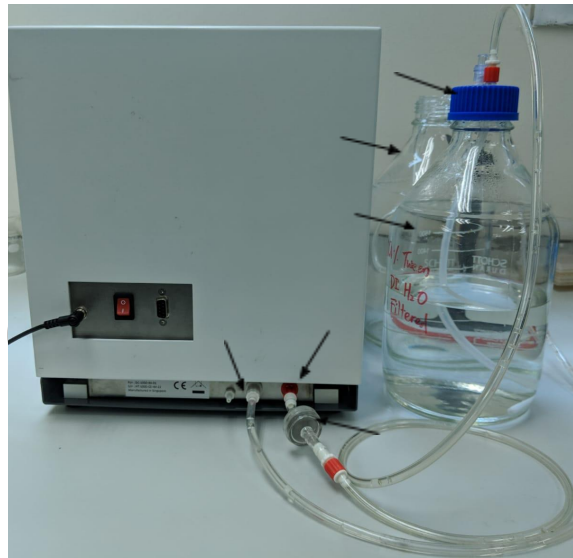


Figure 3-12: Installation Step 3.

Buffer Inlet Bottle Cap Installation

NOTE: The buffer inlet bottle cap must not be used on the waste bottle.

1. 3 sets of buffer inlet bottle caps are packed with the machine. Each bottle cap comes with a pre-installed tubing, as shown in Figure 3-2 below.



Figure 3-13: Buffer inlet bottle cap accessories

2. Connect a buffer inlet bottle cap onto a 2L glass bottle containing the wash buffer of choice, as shown in Figure 3-3 below.

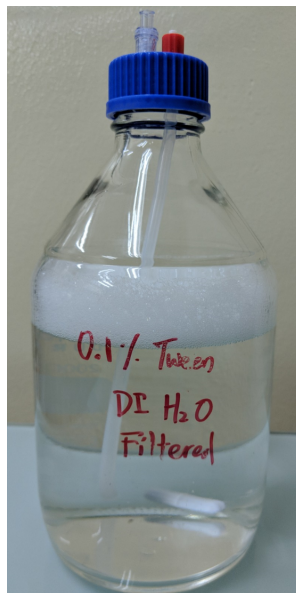


Figure 3-14: Buffer inlet bottle cap connected to 2L bottle containing wash buffer of choice.

16. Install a clean filter onto one end of the inlet tubing and insert into the red port on the HT2000. Insert the other end into wash buffer bottle via the buffer inlet bottle cap. Refer to Figure 3-12.

Preparation for Operation

It is mandatory to perform priming of the machine with 1% Tween 20 in 70% Ethanol, followed by 1% Tween 20 in DI water and finally with Wash Assay Buffer at the start of the day's operation. A dummy wash is performed after priming for added assurance that the machine is performing to specifications prior to an actual wash. Follow the steps below to prepare the machine for operation.

If the machine installation will be carried out in a cold room, or moved into a cold room, acclimatize the machine in the cold room for at least 3 hours. Proceed to the following calibration steps afterward. It is recommended to do a weekly calibration test to ensure optimal performance and recalibration will be required after moving the machine out from the cold room.

1. Ensure the power jack is connected. Power up the HT2000 and wait for the homing sequence to complete.
2. Enter Service Mode by tapping on "SERVICE MODE". Go to page 2 of 3 tab by tapping on [←▶]

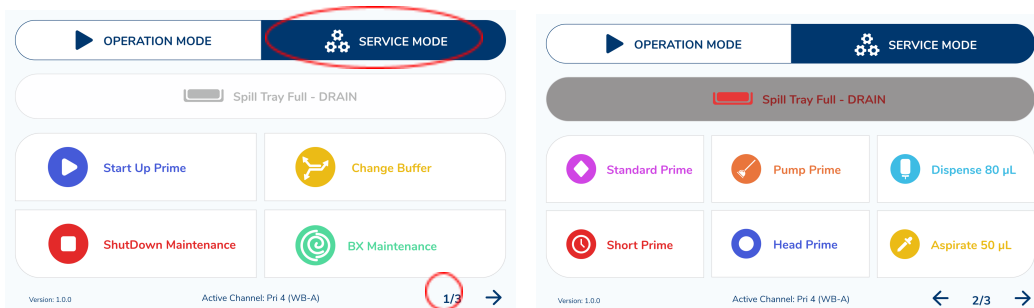


Figure 3-15, 3-16: Enter the Service Mode

3. Ensure the two delivery tubes are properly connected into the respective ports (red and white) on the HT2000. A clean filter is installed into one end of the inlet tubing and inserted into the red port on the machine. The other ends of the tubes are connected to either the wash buffer bottle via the buffer inlet bottle cap (from red port) or the waste container (from white port). Refer to Figure 3-12.
4. Ensure that each bottle is filled with the appropriate fluid, connected to the buffer inlet bottle cap and properly installed into the machine. Refer to "Buffer Inlet Bottle Cap Installation" on page 20.
5. Ensure 1% Tween 20 in 70% Ethanol is connected to the machine.
6. Place a dummy plate onto the feeder platform.



Figure 3-17: Insert dummy plate into machine (in Service Mode)

Preparation for Operation | 25

7. Tap on “Standard Prime” to perform the auto-prime with 1% Tween 20 in 70% Ethanol process.

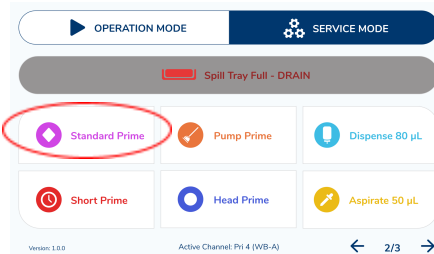


Figure 3-18: Auto-prime (in Service Mode)

8. HT2000 completes the auto-prime with 1% Tween 20 in 70% Ethanol when the feeder platform moves out the dummy plate.
9. Repeat steps 5 to 8, replacing fluid to 1% Tween 20 in DI water.
10. Replace the dummy plate with a calibration plate.
11. Tap on “Dispense 80µL” button to dispense 80µL 1% Tween 20 in DI water into all wells.

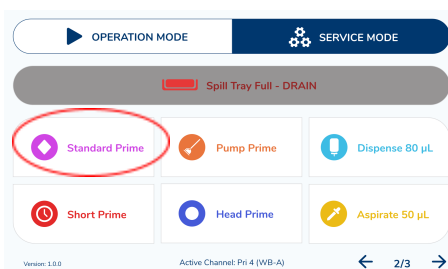
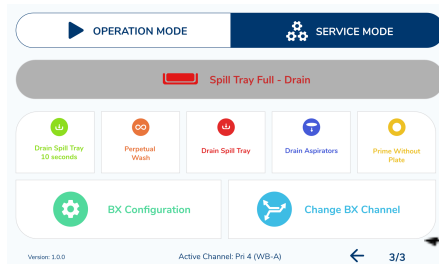


Figure 3-19: dispense 80µL (in Service Mode)

12. Remove the calibration plate from the feeder platform.
13. Visually observe that all wells are dispensed evenly and weigh on a calibrated balance.
14. Weight of empty plate is subtracted from weight of filled plate.

- Resultant value is divided by number of wells to obtain volume dispensed per well, with the assumption that $1\text{mg} = 1\mu\text{L}$ and that all wells are filled evenly.
- Go to “Service Engineering” for adjustment. Tap on “Calibrate Disp Vol” to input the calculated value into the system and then update it by tapping on “Update”.



Tap 5 times continuously at this corner to enter “SERVICE ENGINEERING”

Figure 3-20: Calibration (in Service Mode)

26 | Chapter 3: Preparation for Operation

- Tap on “Calibrate Disp Vol” to input the calculated value into the system and then update it by tapping on “Update”.

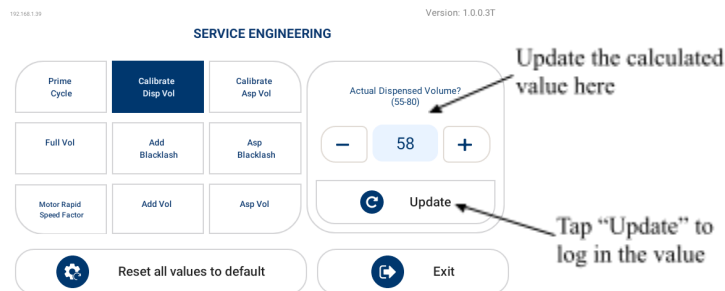


Figure 3-21: Calibration (in Service Engineering)

- Repeat steps 11 to 17 until $80\mu\text{L}$ is achieved.
- Tap on “Exit” to exit Service Engineering tab.
- Tap on “OPERATION MODE”.
- Input the number of washes value as “1”, the initial volume value as “80” and flow rate as “5” into the system by using the “+” or “-” symbols.

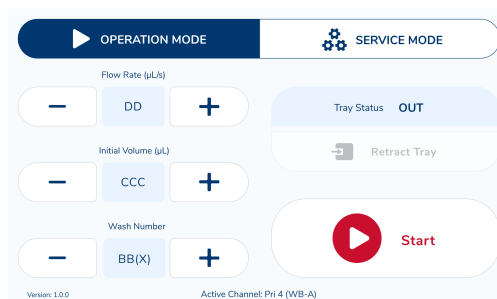


Figure 3-22: Input parameters (in Service Mode)

- Press the “Start” button to start the wash cycle.
- Remove calibration plate from the feeder platform.

24. Visually observe that residual volumes on all wells are even and weigh on a calibrated balance.
 25. Weight of empty plate is subtracted from weight of filled plate, then divided by the number of wells to obtain residual volume per well, with the assumption that 1mg = 1μL and that the residual volume on all wells are even.
 26. Repeat steps 5 to 8, replacing fluid to buffer of choice (e.g. FACS buffer).
 27. The machine is now ready for operation.
-

NOTE:

1. *If the subsequent washing requires sterile environment, the dummy plate should be sterilized by rinsing with 70% EtOH and drying under UV before use*
 2. *If the HT has been under prolonged storage, refer to Appendix C for instructions on how to requalify the machine*
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Chapter 4:

Operation

Chapter Overview

- Before Operating the HT2000
- Operational Safety
- Operation Mode
- Service Mode
- Shutdown Procedure

Before Operating the HT2000

The HT2000 should be placed on a flat surface and in an environment with temperature and humidity as stipulated in “Technical Specifications” on page 3.

Operational Safety

The HT2000 possesses the following built-in safety features which prevent the machine from operating under unsafe conditions.

Plate Detection

The HT2000 detects the presence of a Laminar Wash 96-well Plate. The machine will not begin operation if a plate is not detected on the feeder platform.

Power Supply

The universal power adapter is able to accommodate electrical power in the range of 100 - 240 V. The resultant output to the HT2000 power jack is 24 V DC.

Emergency Stop

Activating the emergency stop button in the middle of a washing cycle will cause the machine to immediately freeze. The machine goes to home position when the emergency stop button is subsequently deactivated.

Machine Homing

In the event power to the machine is cut off in the middle of a washing cycle and subsequently the machine is switched on again, the machine will go into home position.

Spill Tray

The spill tray sensor detects excess liquid in the tray to prevent it from spilling onto the machine's interior. When this happens, the machine LED light turns red and error code “E31” is prompted on the machine display panel. User may only access the draining button during this time.

Safety Precautions

In addition to the built-in safety features of the HT2000, below are some precautions operators are advised to take while using the HT2000 to ensure their safety and to preserve the accuracy of the experiments.

- At the end of a wash process, remove the plate from the fluidics chamber immediately to prevent the wells from drying, stressing cells and/or deteriorating biological integrity.
- Keep the area around the power supply free from liquid.
- During active operation, keep your hands off the instrument other than using the display panel.
- If there is an unexpected error, reset the instrument by turning the power switch off and back on.

Operational Mode

The Operation Mode allows an operator to set up parameters as well as washes.

User Interface

- ***User Interface LCD***
The user interface shows 3 values: “Flow Rate”, “No. of Washes” as well as “Initial Volume”.
- ***“Start” button***
Press the “Start” button to initiate full wash cycle. When pressed mid-operation, it will stop the wash cycle immediately and extend the feeder platform, returning the plate.
- ***“Flow Rate”***
Flow rate of dispensing and aspirating fluid in ul/s. The value can be adjusted by tapping the ‘+’ or ‘-’ sign beside the value. The lower and upper limit is 2 and 20 respectively.
- ***“Initial Volume”***
Initial volume of droplet on each well in the plate in ul. The value can be adjusted by tapping the ‘+’ or ‘-’ sign beside the value. The lower and upper limit is 1 and 99 respectively.
- ***“Wash Number”***
Self-explanatory. The value can be adjusted by tapping the ‘+’ or ‘-’ sign beside the value. The lower and upper limit is 1 and 19 respectively.
- ***“Tray Status”***
Indicate if the tray is ‘Retract’ or ‘Extend’

- **“Version”**
This indicates the current version of the Graphical User Interface.
- **“Active Channel”**
This indicates the channel that is currently activated, if connected to a buffer exchanger.

Function Buttons

- **[Retract] or [Extend]**
To move the feed tray in or out of the washer.
Note: feed tray cannot be retracted if a plate is detected on it
- **[Start]**
To begin a wash using the wash parameters as displayed in this tab.
- **[SERVICE MODE]**
Tap to switch to Service tab.

Wash Cycle

1. Feeder platform detects for presence of plate and retracts into the machine.
2. Fluidics head lowers down into plate.
3. Based on indicated initial volume, dispensing head tops up well contents until 80µl (e.g. if initial volume is 50µl, dispensing head adds 30µl to the droplet).
4. Aspirating head aspirates full volume based on internal firmware. It is intended that contact between aspirating nozzles and well droplet breaks before aspiration is completed. Once the aspiration nozzle completes the aspiration of existing drops at a given height of a nozzle, it continues to aspirate air. Resultant residual volume should be approximately 25µl based on the height of an aspiration nozzle.
5. Dispensing head tops up well contents based on internal firmware.
6. Steps 4 and 5 are repeated until stipulated number of washes is reached. Note: if only 1 wash is specified, the machine will only run until step 4.
7. Feeder platform extends out of the machine, returning plates with approximately 25µl residual volume in each well.
8. The wash cycle of 1x dispensing and aspiration consumes ~6 ml wash solution. For example, 5x wash cycle consumes ~30ml wash solution.

Service Mode

The Service Mode allows an operator to carry out priming, cleaning, draining, and changing channels operations.

User Interface

- **User Interface LED**

The user interface in Service Mode will show 3 pages of functions

- **“Version”**

This indicates the current version of the Graphical User Interface.

- ❖ **“Active Channel”**

This indicates the channel that is currently activated, if connected to a buffer exchanger.

- ❖ **[OPERATION MODE]**

Tab to switch to Operation Mode

- ❖ **[Spill Tray Full – Drain]**

This tab is usually greyed out and inactive. However if the spill tray full sensor is activated, all other functions in the Service tab will be greyed out, with only this tab active. User must press this tab continuously to activate pump to remove fluid in the spill tray.

- ❖ **[→▶]**

Flip to the next page

- ❖ **[◀←]**

Return to previous page

Function tab (1st of 3 tabs)

- ❖ **[Start-up Prime]**

Enter the guided packaged function to start-up the washer by priming with ethanol, followed by water, and if indicated, followed by selected wash buffer.

- ❖ **[Change Buffer]**

Enter guided packaged function to switch wash buffer.

- ❖ **[Shut Down Maintenance]**

Enter guided packaged function to prime water, followed by ethanol, and finally by air to prepare washer for shutdown.

Note: For the 3 packaged functions above, ‘Short Prime’ are utilized for the prime functions. Each prime cycle will use approximately 130ml of fluid. See function [Short Prime] for more information.

❖ **[BX maintenance]**

To select BX for flushing its channels. The function will sequentially run fluid through all the channels in the selected BX, each activated for 10 seconds.

Note: The flushing means the fluid will pass through the washer inlet, through the dispensing head reservoir, the pump, and finally out of the washer outlet.

Function tab (2nd of 3 tabs)

❖ **[Standard Prime]**

Perform a standard prime by the washer. A plate is required to be present for this function to be activated. The operation will consume approximately 180ml of fluid, and will achieve greater than 100X dilution of the prior fluid.

❖ **[Short Prime]**

Perform a short prime by the washer. A plate is required to be present for this function to be activated. The operation will consume approximately 130ml of fluid, and will achieve approximately 40X dilution of the prior fluid.

❖ **[Pump Prime]**

Run the pump only to prime the head for duration of 2 seconds. A plate is required to be present for this function to be activated. Multiple sequential runs can pump fluid into the reservoir of the dispensing head, but it will not run the fluid through the nozzles. Hence this function cannot be used as a substitute for Standard Prime or Short Prime. However, if a set-up requires an inlet tubing that is too long for Standard or Short Prime to properly fill the reservoir on the dispensing head, Pump Prime may be activated prior to Standard or Short Prime to pre-fill the some air gap.

❖ **[Head Prime]**

Run the dispensing head for 5X, followed by aspirating head for 7X. A plate is required to be present for this function to be activated. The function runs the fluid from the dispensing head reservoir through the nozzle. Unlike Standard Prime and Short Prime, this function is not preceded by pump activation that pulls fluid into the dispensing head reservoir.

Note: In case that the pump in the washer fails, this function can act as an emergency prime function, albeit there will be higher wash buffer wastage in the process.

❖ **[Dispense 80ul]**

Dispense 80ul of fluid onto each well on the plate. A plate is required to be present for this function to be activated. This function is use for checking the working status of the dispensing head.

❖ **[Aspirate 50ul]**

Aspirate 50ul of fluid from each well on the plate. A plate is required to be present for this function to be activated. This function is use for checking the working status of the aspirating head.

Function tab (3rd of 3 tabs)

❖ [Drain Spill Tray 10 seconds]

Tap once to activate this function. Will run the pump to drain fluid from the spill tray for 10 seconds.

❖ [Perpetual Wash]

Tap once to activate. A plate is required to be present for this function to be activated. This function will run wash cycle continuously until user disrupt the process.

❖ [Drain Spill Tray]

Press continuously to activate. Will run pump to drain the spill tray as long as the tab is pressed. The process will end once the touch is lifted.

❖ [Drain Aspirator]

Tap once to activate. Aspiration block will cycle up/down for 3 times, followed by 2 seconds of pump aspiration.

❖ [Prime Without Plate]

Press continuously to activate. While pressed, the pump will continue to prime fluid into the head. Upon release, the dispensing head will cycle up/down 3X before ending the function. Fluid that ran through the nozzles will be collected in the spill tray.

Note: If the spill tray was previously filled with wash buffer, this function can be used to fill the spill tray with water or ethanol for the purpose of cleaning it. The water/ethanol can subsequently be drained off the spill tray using [Drain Spill Tray 10 seconds] or [Drain Spill Tray].

❖ [BX Configuration]

This tab is to enter the page to inform the washer of the Buffer Exchanger configuration. Certain process & option in the service tab may change according to the configuration selection.

Important note 1: If the HT2000 is connected to a buffer exchanger, do not set the BX Configuration selection as 'None'. A 'None' selection will dictate that none of the BX channel is selected, thereby cutting any fluid flow path to the washer. This situation can cause extreme stress on the fluidic head on the washer, and may cause irreversible damage to it.

Important note 2: For the same reason as above, the appropriate cable must be inserted into the correct electrical ports that link the HT2000 washer to the BX, to ensure the appropriate channel is activated. Otherwise damage to the washer head may occur.

❖ [Change BX Channel]

Make an immediate switch to the selected channel.

Note: [Change BX Channel] is not the same as [Change Wash Buffer]. [Change Wash Buffer] will activate a channel switch, followed by a [Short Prime] to ready the washer with new wash buffer. [Change BX Channel] will only switch the channel, but do not prime the washer.

Calibration

It is recommended to perform volume calibration of the machine during new machine installation, quarterly and after long term storage. Refer to page 24, step 11 to 17 to calibrate the machine.

Shutdown Procedure

It is recommended to perform priming of the machine with 1% Tween 20 in DI water, followed by 1% Tween 20 in 70% Ethanol and finally without fluid at the end of the day's operation. Follow the steps below to prepare the machine for operation.

1. Ensure the power jack is connected. Power up the HT2000 and wait for the homing sequence to complete.
2. Enter Service Mode by tapping the "Service Mode"
3. Ensure the two delivery tubes are properly connected to their respective ports (red and white) on the HT2000. A clean filter is installed into one end of the inlet tubing and inserted into the red port on the machine. The other ends of the tubes are connected to either the wash buffer bottle via the buffer inlet bottle cap (from red port) or the waste container (from white port). Refer to Figure 3-1.
4. Ensure that each bottle is filled with the appropriate fluid, connected to the buffer inlet bottle cap and properly installed into the machine, as per "Bottle Cap System Installation" on page 20.
5. Ensure 1% Tween 20 in DI water is connected to the machine.
6. Place the dummy plate onto the feeder platform.
7. At page 2 of "Service Mode", press Standard Prime to perform the auto-prime with 1% Tween 20 in DI water process.
8. HT2000 completes the auto-prime with 1% Tween 20 in DI water when the feeder platform moves out the dummy plate.
9. Repeat steps 5 to 8, replacing fluid connection to 1% Tween 20 in 70% Ethanol.
10. Repeat steps 5 to 8, replacing connection to air intake only, refer to Figure 3-4.
11. Power down the HT2000 and prepare for storage.

Chapter 5:

Maintenance and Troubleshooting

Chapter Overview

- Maintenance Schedule
- Common Problems for Troubleshooting
- Technical Support

Maintenance Schedule

Below is the recommended maintenance schedule to ensure that the HT2000 runs smoothly and efficiently.

Action	Daily	Weekly	Monthly	As Required
Perform priming of the machine with 1% Tween 20 in 70% Ethanol, followed by 1% Tween 20 in DI water and finally with Wash Assay Buffer at the start of the day's operation.	✓			
Perform priming of the machine with 1% Tween 20 in DI water, followed by 1% Tween 20 in 70% Ethanol and finally without fluid at the end of the day's operation.	✓			
Wash Filter under running DI water on a weekly basis.		✓		
Bleach run for cleaning of internal fluidics: 1. Enter Service Mode and initiate perpetual wash with 0.5 % Bleach for <u>strictly</u> 10 minutes. 2. Run perpetual wash with 1% Tween 20 in DI water for <u>minimum</u> 20 minutes. Caution: Bleach is corrosive agent, perpetual wash with 1% Tween 20 in DI water should be run immediately after the 10 minutes bleach run. Failure to do so may cause damage to the machine's component.				✓
It is recommended to perform volume calibration during new machine installation, quarterly and after storage for long period. Refer to "Calibration" on page 28.				✓
Sending unit to Curiox Biosystems for service. Decontaminate washer and complete Appendix B.				✓

Common Problems for Troubleshooting

Problem 1: Display panel is not lit up/ Machine has no power/ Failure to initialize/ Motor error

Possible cause: Power is not switched on, or HT2000 is not plugged into electricity supply, or wrong power adaptor is used.

- ❖ Ensure the jack is properly connected to the HT2000, and that the power is switched on.
- ❖ Ensure that the correct adaptor is used and that it is working.

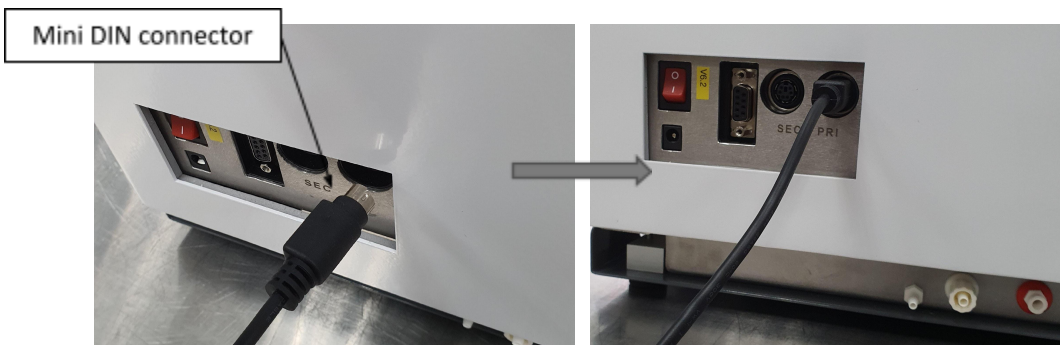
Problem 2: No plate detected

Possible cause: Plate sensor cannot detect the plate properly due to wet feeder tray, or condensation, or wet bottom of the plate.

- ❖ Ensure that the bottom of the plate is clean and dry
- ❖ Ensure that the feeder tray is clean and dry

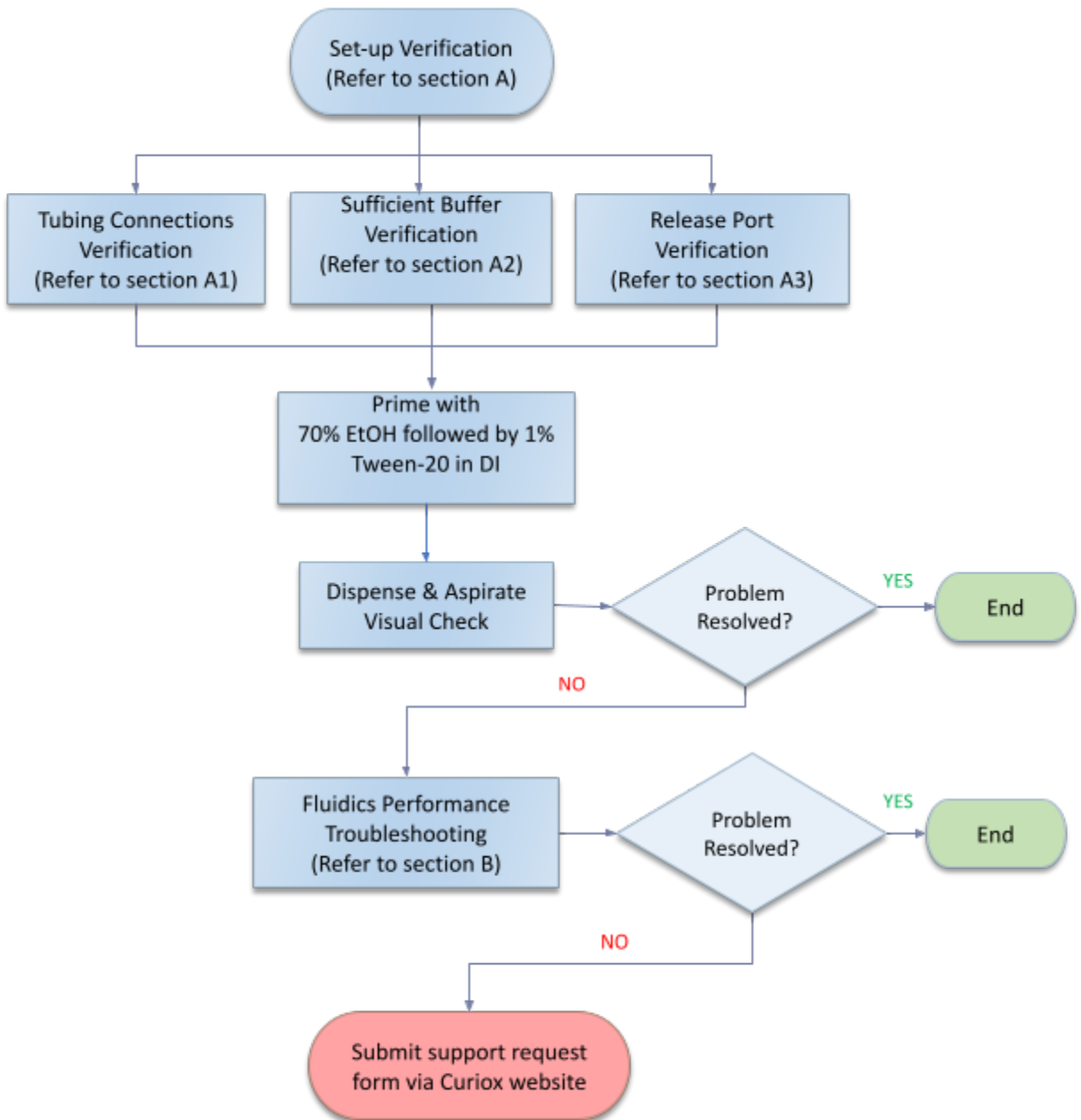
Problem 3: Failure to dispense and aspirate properly/ Washer not drawing buffer/ Inconsistent residual volume

- ❖ If buffer exchanger is used, ensure that the Mini DIN connector is fully inserted and connected properly. A subtle click sound can be heard when changing between the different channels on the buffer exchanger.



- ❖ If the problem persists, proceed with the following troubleshooting.

- ❖ Refer to the flowchart below for the overall troubleshooting process. Perform set-up verification followed by fluidics performance troubleshooting as described in the corresponding sections A and B to resolve the problem.



A Set-up verification:**A1) Tubing connections verification**

Check for any signs of leaking on the machine's inlet and outlet tubing. Also, check the tubing connections for any leakage. Where leakage is present, disassemble and carefully reassemble the affected tubing connection.

A2) Sufficient buffer verification

Verify that buffer being supplied to the machine is sufficient and that tubing is completely submerged in buffer.

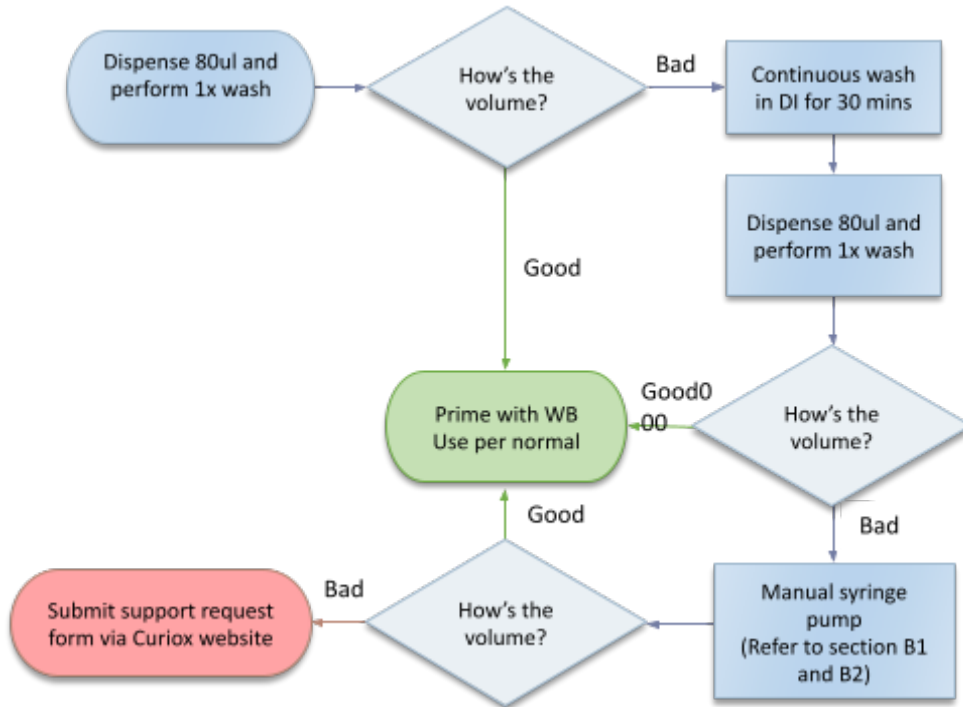
A3) Release port verification

Verify that the release port located on the bottle cap is not blocked.



A4) Perform standard prime with 1% Tween-20 in 70% EtOH followed by 1% Tween-20 in DI water. Do a dispense and aspirate visual check to see if the issue is resolved. Where inaccuracies are dispensed and aspirated volume is still observed, proceed to the next section for fluidics performance troubleshooting.

B Fluidics performance troubleshooting flowchart

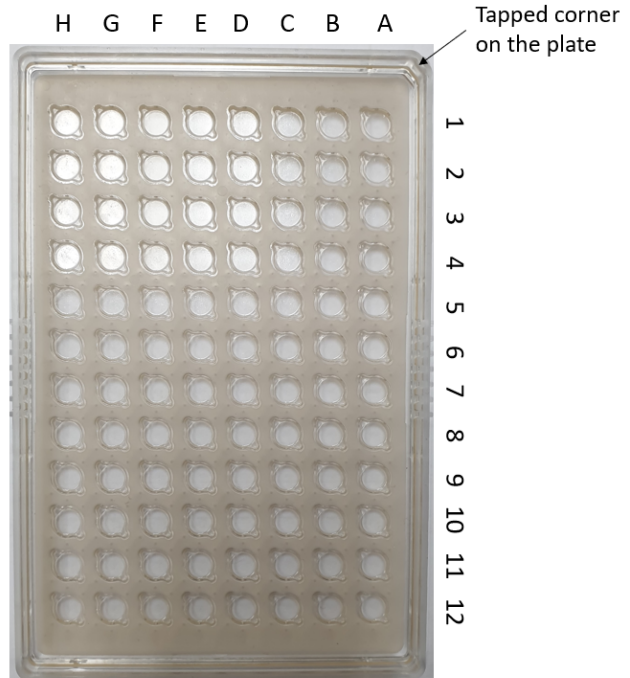


B1) Identify the affected dispensing and aspirating nozzles corresponding to the affected well(s) using the plate and nozzle mapping.

B1.1 Plate mapping:

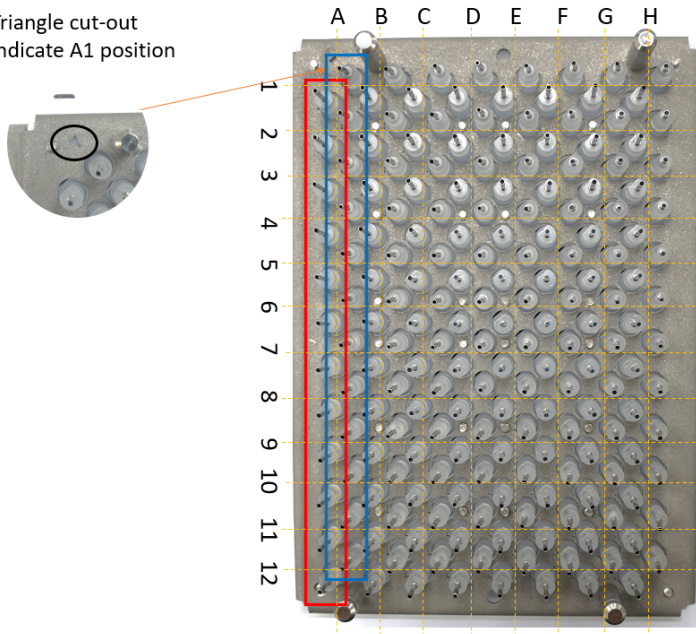
Locate affected well according to this plate mapping

Refer to nozzle mapping to test the affected nozzle



B1.2 Nozzle mapping:

Triangle cut-out indicate A1 position



Red Box → Dispensing Nozzle (Straight Needle)

Blue Box → Aspirating Nozzle (Bent Needle)

Locate the intersection of the affected well/s.

Dispensing nozzles are located to the bottom left of the intersection point.

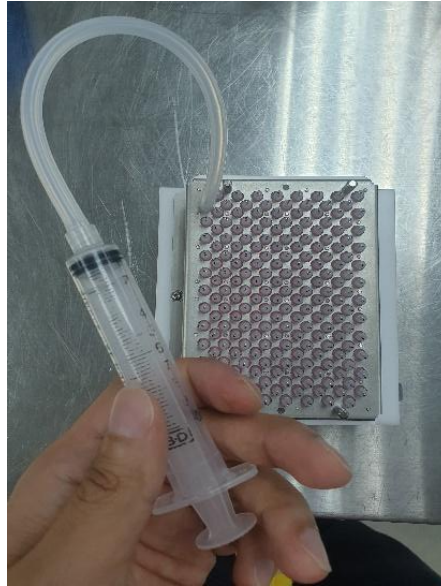
Aspiration nozzles are located to the top right of the intersection point.

B2) Execute manual syringe pump

B2.1 Prime the fluidic head with air to remove any liquid from the fluidic pathways.

B2.2 Take out the fluidic head from the washer and turn it over.

B2.3 Fit the tubing connected with the syringe to the affected dispensing nozzle.



B2.4 Pull the plunger fully to the back. There should be minimal resistance.

Note: If there is significant resistance, there is clogging. Please submit a support request form via Curiox website

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B2.5 Try to push in the plunger. You should feel resistance upon pushing the plunger.

B2.6 Remove the tubing from the dispensing nozzle.

B2.7 Pull the plunger fully to the back to fill the syringe with air.

B2.8 Fit the tubing connected with the syringe to the affected aspirating nozzle.



B2.9 Push the plunger fully into the syringe. There should be minimal resistance.

Note: If there is significant resistance, there is clogging. Please submit a support request form via Curiox website

request form via Curiox website.

- B2.10 Try to pull the plunger. You should feel resistance upon pulling the plunger.
- B2.11 Use the head spacer to ensure that the three blocks of the fluidic head are aligned and slot the fluidic head into the washer (For installation of fluidic head refer to Chapter 3, pg. 18-21).
- B2.12 Perform a standard prime with 1% Tween-20 in 70% EtOH.
- B2.13 Perform a standard prime with 1% Tween-20 in DI water.
- B2.14 Do a dispense and aspirate visual check to see if the issue is resolved. If the problem persists, please submit a support request form via Curiox website.

Technical Support

Kindly visit us at www.curiox.com to submit a support request form or email us at sales@curiox.com if you require technical support or advice.

Appendix A:

Acknowledgment of Decontamination

Acknowledgment of Decontamination Form

Decontamination is required prior to HT2000 return to Curiox Biosystems for reasons such as servicing and maintenance. It is required that you fill out this form to acknowledge that decontamination had been conducted on the instrument. Failure to do so may result in the return of the instrument to your address for decontamination.

PRODUCT SERIAL NO.		
CONTACT INFORMATION		
Dr./Mr./Mrs./Ms. (Please circle accordingly)	JOB TITLE	
NAME	EMAIL ADDRESS	
COMPANY	PHONE NUMBER	
DECONTAMINATION INFORMATION		
DECONTAMINATION METHOD		
DECONTAMINATION DATE		
ACKNOWLEDGEMENT		
<p>I hereby acknowledge that this piece of equipment has been decontaminated and sealed in accordance to the procedure recommended in this manual prior to shipment to Curiox Biosystems. To the best of my knowledge, the equipment is safe to handle by the receiving personnel.</p>		
<p>_____</p> <p>Name</p>		
<p>_____</p> <p>Signature and Date</p>		

Appendix B:

Purchase Information and

Feedback Form

Purchase Information and Feedback Form

PURCHASE INFORMATION	
PRODUCT SERIAL NO.	
PURCHASED BY	PURCHASED FROM
COMPANY	DISTRIBUTOR
ADDRESS	DATE OF PURCHASE
PHONE	DATE OF DELIVERY
FAX	
CONTACT INFORMATION	
Dr./Mr./Mrs./Ms. (Please circle accordingly)	JOB TITLE
NAME	EMAIL ADDRESS

FEEDBACK (PLEASE CHECK/COMMENT ACCORDINGLY)					
	Excellent	Good	Average	Poor	Comments
User Guide					
Ease of Use					
Reliability					
Operating Costs					
Overall Experience					

Do you have any other comments/suggestions about the Laminar Wash™ HT2000? If so, please let us know below.

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