BioFluidica™ LiquidScan®

User Manual

05/2023 – Revision B.

BioFluidica LiquidScan® User Manual

Disclaimer

For Research Use Only. Not for diagnostic procedure

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Introduction

1.1 About this Manual

This LiquidScan User Manual provides instructions on the operation of the LiquidScan System on a Hamilton STARlet automated liquid handler. Read through the entirety of this manual before operating the system.

This manual will cover the required startup procedures, deck setup, labware, consumables, and instructions for using the LiquidScan software.

1.2 Intended Use of the LiquidScan System

The LiquidScan System is an automated liquid handling platform. Along with reagents and specific chip sets, it allows for the enrichment of rare cells and extracellular vesicles from biological or artificial fluids. The enriched samples are released and available for downstream processing and analysis. Downstream processing and analyses are outside the scope of this manual and will be determined according to the needs of the laboratory.

1.3 Disclaimer

This manual provides instructions for use in a 16-channel LiquidScan STARlet. Customized training may be necessary for use in different models of the Hamilton STAR line of liquid handlers.

This manual is not intended to instruct the user in operating the VENUS software of the Hamilton Microlab STAR line of robotics.

BioFluidica is not responsible for any deviation, inaccurate, or false outcomes caused by procedures outside of the scope of this manual. The use and operation of the LiquidScan System indicates user acknowledgment of all warnings and stipulations contained within the manual, which releases any liability by BioFluidica.

Critical and important warnings and notifications in this manual will be proceeded with "**Important**" and italicized.

Under licensed permissions, LiquidScan System steps, workflows and altered deck setups are available to users. Contact BioFluidica if custom applications are needed for the laboratory.

The content of this manual is subject to change without notice. Version numbers are found on the front cover of this manual. Contact BioFluidica if any errors, unclear points, or mistakes are found in this manual.

1.4 Software End User License Agreement

The copyright on the software and supplied documentation belongs to BioFluidica. The user holds the license for its intended use only. The user may not copy LiquidScan Software nor sell or distribute to a third party.

BioFluidica is not liable for any damages arising from the use of the software by the user or a third party.

BioFluidica may terminate and cancel the contract if the user fails to comply with the terms of the Software Licensing Agreement. If non-compliance is found, BioFluidica has the right to request the user to return or destroy the software and all copies of BioFluidica documents.

If custom methods are needed, contact BioFluidica to program the methods. BioFluidica is not responsible for damage to the system or results from methods not created by BioFluidica.

However, with the proper user access, the LiquidScan software can be programmed by the user to perform methods and workflows that differ from those installed by BioFluidica. Contact BioFluidica to obtain additional training.

2

LiquidScan® System Components

2.1 LiquidScan System Components

The LiquidScan System consists of the following parts:

1. LiquidScan STARlet
2. LiquidScan Module
3. LiquidScan Kits
4. LiquidScan Software

The user must supply associated consumables. Users must use original parts commercially available by the listed suppliers:

Product Name	Catalog Number	Supplier
1000 µL Conductive Tips (3840 tips)	235904	Hamilton
300 µL Conductive Tips (5760 tips)	235902	Hamilton
Biohazard waste bags (25 bags)	199203	Hamilton
USER Enzyme + CutSmart Buffer (250 U)	M5505L	New England Biolabs
BioFluidica LiquidScan Kit Options		
BioFluidica Blood Collection Kit	3000415	BioFluidica
CTC - Solid Tumor Kit - 8 chips	3000418	BioFluidica
CTC - Solid Tumor Kit - 4 chips	3000414	BioFluidica
Fetal Cell - Prenatal Kit - 8 chips	3000428	BioFluidica
Fetal Cell - Prenatal Cell Kit - 4 Chips	3000424	BioFluidica
Custom Antibody Kit – Sinusoidal Chips	3000425	BioFluidica
Custom Antibody Kit – Pillar Chips	3000426	BioFluidica

2.2 LiquidScan System Documents Provided

- LiquidScan User Manual
- LiquidScan Quick Start Guide
- Certificate of Analysis for the Kit
- Safety Data Sheet (SDS) for the Kit

2.3 LiquidScan System Package Verification

While the LiquidScan System package may vary based on the Terms and Conditions between the user and BioFluidica, below are the general components of the system.

Before use of the LiquidScan System, inspect and verify that all components are in place. In case of defective or broken items, contact BioFluidica.

LiquidScan STARlet	
LiquidScan Module	
LiquidScan Kit – 8 chip kit • Tube with preservative • 8 x Microfluidic Chips (Packaged in foil) • 1 x Reagent Plate • 1 Tube for USER • 8 Elution Tubes • 8 Waste Tubes B^3 Blood Collection Kit	
LiquidScan Software	Installed by BioFluidica Field Application Specialist.

3

Safety Precautions

3.1 Safety Labels and Precautions

	**Biohazard Warning** Deck contains biohazardous materials.
	**Pipetting Arm** **Connection to PC** **USB Connection** **Moving Parts**
	**Hot Surface**
	**LED Light**
	**Power Connection**
	**Keep Hands Clear when equipment is running**

	**Strong magnet. Keep hands clear**

3.2 Operational Safety Requirements

Before using the LiquidScan System:

- Read Microlab STAR Line Operator's Manual.
- Read all Safety Data Sheets (SDS) regarding proper storage, handling, transport, and disposal of chemicals used.
- Wear appropriate Personal Protective Equipment (PPE) according to laboratory standards.
- Follow applicable policies and regulations regarding the proper handling, transport, and disposal of any biohazardous materials.

The LiquidScan System does not include an uninterruptible power supply (UPS). Use of a UPS is recommended. Any interruption of power supply may result in data or sample loss.

The orange cover on the LiquidScan Module has a strong magnet to secure chips during system operation. Exercise caution when opening and closing the lid as obstructing the lid's movement can result in pinched fingers or hands.

Important: *Placement of any unapproved labware or material on the deck may result in a collision of the pipetting channel heads and or arm resulting in damage to the system.*

Failure to insert labware properly within the rail system can lead to canting of the labware and collision of pipette heads during XY plane movement, causing damage to the channels. Failure to place consumables within their respective carriers can cause collision and damage to pipetting heads.

The system contains a front cover that should be closed while the instrument runs. During the operation of the LiquidScan system, keep hands out of the movement pathway of the pipetting arm.

Users should stand clear of the deck and any moving parts during system operation. BioFluidica nor Hamilton are responsible for injury or accidental damage to the system due to inappropriate or improper placement of labware or body parts within the system.

Important: *Do not lean on the loading tray. Never put any portion of body parts into the deck space while the system is in use. Severe personal injury may occur.*

3.3 Biological Hazard

Be careful of biohazard and chemical contamination. In the event of a spill or contamination of the system with biohazardous or chemical materials, it should be cleaned according to the user's institution's policies.

Always wear proper Personal Protective Equipment (PPE) during the decontamination procedure. Decontaminate any portion of the LiquidScan System that encounters contaminants.

Do not use any disinfecting materials that contain hypochlorite on LiquidScan STARlet. 70% ethanol may be used on stainless steel surfaces or other surfaces that are incompatible with bleach.

3.4 Electrical Safety

Turn off and disconnect LiquidScan and Hamilton STAR instruments from all electricity before moving or adjusting the electrical components.

Electromagnetic radio frequency (RF) and/or static electricity may negatively affect liquid level detection in the system. Be sure to keep equipment that may emit electromagnetic RF away from the system and minimize the potential of static electricity within the environment around the instrument. Electrostatic discharge can affect both labware and the liquid level detection of the system. Avoid any electrostatic charge to any portion of the instrument or tips when operating the system.

3.5 Transport and Installation

Hamilton installation/field technicians are responsible for the installation qualification (IQ) and operation qualification (OQ) of the Hamilton STAR instrument line. If the Hamilton STARlet is transported and re-installed, it is the user's responsibility to contact Hamilton to recalibrate the instrument.

The BioFluidica Field Application Specialist (FAS) is responsible for installing the LiquidScan software, LiquidScan Module, the associated power cord, and the control box. All elements will be installed in a manner that prevents interference with the robotic arm of the system.

To ensure safe use of the system, the BioFluidica FAS will provide onsite training. Please contact BioFluidica to arrange any additional training.

Important: *Deviation from the procedures herein may lead to inaccurate or false results.*

3.6 Product Disposal

All components found in the LiquidScan Kit can be discarded according to laboratory policies.

The LiquidScan Module and LiquidScan STARlet must be disposed of according to the local regulations for potentially hazardous waste and electronic equipment.
Please contact the legal offices in your local area or contact BioFluidica for further assistance.

4

Component Functions

4.1 System Description

The LiquidScan® System consists of two hardware components: the LiquidScan STARlet and the LiquidScan Module.

The LiquidScan STARlet is a Sequential Transfer and Aliquoting Robot (STAR) controlled by a program known as Venus. The robot's purpose is to perform pipetting and aliquoting operations of liquids between containers.

The robot can be purchased with either 8-channel or 16-channel configurations. The channels are numbered from back to front on the pipetting arm. When facing the system, channel 1 is located at the rear of the instrument, and channel 8 or 16 is closest to the user.

By itself, the robot cannot perform the complicated task of sample enrichment. The robot must be paired with the LiquidScan Module and LiquidScan Software and use a LiquidScan Kit to accomplish this task. All the components work together to ensure a no-loss sample transfer and enrichment process.

4.2 LiquidScan STARlet

***Important**: When discussing any Hamilton labware, the term labware refers to some form of a carrier that houses either tips or other plasticware, such as microcentrifuge tubes, sample tubes, reagent plates, etc. When referring to positions on a labware carrier, the first position is always at the rear of the instrument, and the position number increases as the user moves toward the front of the instrument. Mention of carrier designations refers to Hamilton's internal naming system for their labware carriers, e.g., SMP_CAR_24_A00. This designation is located on a black sticker on the front of the carrier.*

The robot's main work surface is called the deck and contains rails, also known as tracks. The STAR line of robots consists of 30 tracks (STARlet) or 55 tracks (STAR). On the loading tray and the deck proper, there are small black separators, known as slide blocks, to hold and help slide labware carriers into place. Labware carriers have rails that slide into tracks located on both the deck proper and the loading tray. Labware carriers used on the LiquidScan robot either reside on a width of one track (1T labware) or six tracks (6T labware). Before sliding the carriers onto the deck proper, all setup of labware should take place on the loading tray. The loading tray has small black tabs called labware stops that are in the autoload movement area to prevent the movement of carriers onto the deck. The labware stop must be depressed to push the carrier onto the deck.

***Important**: Slide Hamilton carriers onto the loading tray until they contact the labware stop. Once all necessary labware is placed on the loading tray, place all plasticware, reagents, samples, etc., into their correct positions. Once all necessary components for a workflow have been correctly placed, then depress respective labware stops to push labware onto the deck. Do not reach into the deck to set up anything. Do everything on the loading tray.*

4.3 LiquidScan STARlet Parts

1. Five-Position Tip Carrier

The tip carrier is a 6-T carrier (designation TIP-CAR_480_A00) for holding up to five 96-tip racks. Please refer to Appendix A for the deck position.

The LiquidScan System uses 1000 µL and 300 µL unfiltered CO-RE tips made by Hamilton. Refer to Appendix B for tip setup and carrier location.

***Important:** Use of tips not manufactured by Hamilton designed specifically for the STAR line of liquid handlers may affect the robot's performance and lead to improper results.*

2. Custom Multiflex Carrier

The multiflex carrier is a 6-T five position multi-purpose carrier (designation APE_CARRIER) for carrying a custom combination of labware. Please refer to Appendix A for the deck position.

For normal operation, Rack 1 and Rack 2 are always used for 300 µL tips only. Rack 5 is a carrier for the LiquidScan reagent plate. The reagent plate will be positioned with A1 in the upper left-hand corner. The BioFluidica sticker and barcode will be facing the right side of the instrument.

3. 24 Position Sample Carrier

The 24 Position Sample Carrier is a 1-T carrier (designation SMP_CAR_24_A00) designed for BioFluidica Blood Collection Tubes or for 17x100mm round bottom tubes, which are supplied with a LiquidScan kit as 15ml round-bottom tube. Refer to Appendix A for positioning on the LiquidScan System.

This carrier contains twenty-four positions for tubes. The LiquidScan System requires three of these carriers to be placed in different tracks on the deck. The supplied 15ml round bottom tubes are placed starting from the top of the carrier position for workflows dealing with multiple tubes. The number of tubes needed for the run corresponds to the number of chips to be processed for multi-tube workflows on Track 14 and Track 15. For example, if 4 chips are used for the run, the user would place four 15ml round bottom tubes in carrier positions 1-4 on the sample carrier.

The first carrier is used for samples and is positioned in Track 14. Refer to Appendix C for sample tube positioning.

The second carrier is used for collecting the sample flowthrough and is positioned in Track 15. Refer to Appendix C for flow-through tube positioning.

The third carrier is used for liquid waste from the run and is positioned in Track 25.A total of eight (8) 15 mL round bottom tubes are placed on this carrier in positions 1-8 regardless of the number of chips being used. This carrier also has a position for a single tube elution workflow. Anytime a single tube elution occurs, plasticware will always be placed into position 17. Refer to Appendix C for waste tube positioning and single tube elution placement.

4. 32-position sample carrier

The 32-Position Sample Carrier is a 1-T carrier (designation SMP_CAR-32_A00). Refer to Appendix A for its positioning on the LiquidScan System. The carrier has 32 positions which contain pre-positioned inserts for microcentrifuge tubes. The LiquidScan System requires two of these carriers.

The first carrier is inserted on Track 16. This carrier requires a single molded tube carrier insert placed in carrier position17, in order to securely position the 0.5mL skirted microtube used for the enzyme preparation. Refer to Appendix C for the position of the enzyme preparation tube.

The second carrier is positioned on Track 26. This carrier contains eight molded tube carrier inserts in positions 1-8 to hold 1.5 mL microcentrifuge tubes. This carrier holds 1.5 mL microcentrifuge tubes that collect the eluates after being processed through the chip. Refer to appendix C for positional patterns on a similar carrier.

Depending on the specific workflow chosen (Multi-Tube Elution), the number of eluate tubes used for the run must match the number of chips used. For example, for a 4-chip run, four microcentrifuge tubes are required. The elution from each chip is sent to its respective position on the elution rack, so chip 1 is sent to carrier position 1, chip 2 is sent to carrier position 2 and so on.

4.4 LiquidScan Module

The LiquidScan module is a 6 track (6-T) carrier for LiquidScan microfluidic chips. Refer to Appendix A for its deck position.

An 8 channel Hamilton STARlet configuration allows for a maximum of 4 chips to be run at a single time. A 16 channel Hamilton STARlet configuration allows for a maximum of 8 chips to be run at a single time.

The LiquidScan module ensures that the microfluidic chips are retained in the proper position and orientation during sample transfer. The front right of the device contains an orange LED BioFluidica logo that illuminates when the module is connected to power. If the logo is not illuminated, read the Troubleshooting Chapter.

The LiquidScan module has an orange lid with a strong magnet to ensure the chips remain in place and are not lifted from the module deck during the run.

Important: *The lid may be difficult for some users to open. Be sure to pull the module onto the loading tray before opening the lid to prevent damage or accidents from occurring.*

The module consists of two separate sides for different cleavage methods. The left side with the copper plating is for the heated enzymatic cleavage method, and the right side of the module is for the photocleavable method (in development). Each side holds one to eight microfluidic chips.

Each chip position on the module contains a set of metal pins to ensure proper placement of the chips on the LiquidScan module.

LiquidScan Module – Heater deck

The front side of the LiquidScan module has a temperature monitoring screen with present value (PV) and set value (SV) indicators. During installation, the SV will be set to 37°C by the BioFluidica FAS. Once installed, the user doesn't need to adjust the temperature. If adjustments are needed, contact BioFluidica.

When the heater is turned on for the enzymatic cleavage method, the PV will increase to reach the SV temperature. The PV may fluctuate ±0.5°C from the SV.

LiquidScan Module – Control Box

The function of the control box is to allow the LiquidScan software to turn on and off both the Heater and LED functions within the software without the need for the User to turn them on manually. The control box is attached to the computer running the instrument with a USB 2.0 cable. It is also plugged into a 12V power source near the instrument. There is a CTRL cable that also connects the LiquidScan module to the control box. This box should never be unplugged from the computer at any time. It should be placed in a safe location out of the way of the operator of the LiquidScan platform.

4.5 LiquidScan Kit Components

Below is the list of components included in the LiquidScan Kit. The type of chips and specific number of items in the kit are dependent on customer order.

1. **B^3 Blood Collection Tube**

   BioFluidica B^3 Blood Collection Tube (BCT) can be used for sample collection and placed directly in the LiquidScan STARlet for sample enrichment. The contents can also be placed into the 15mL round-bottom tubes provided in the kit.

2. **Reagent Plate**

The reagent plate is a 96-deep well 1mL plate. The plate contains all the reagents necessary to run the system. This plate should be stored at 2-8°C. It should be equilibrated to room temperature before use.

3. **Microfluidic Chips**

   Chips are shipped sealed in foil packaging and should be immediately stored at 2-8°C. Equilibrate the chips at room temperature before use.

   Each chip has a specific ID number. The chips are labeled, and extra chip stickers can be found on the package for use at the customer's discretion (ex: documentation, tubes post-processing, etc.) The QR code can be used to scan the ID for laboratory information systems.

   Unused chips that are still wrapped in parafilm can be stored in the refrigerator in a sealed plastic bag with 300 µL of DI water until the expiration date.

4. **15ml Round Bottom Tubes**
   The 15 mL round bottom tubes can be used for samples, sample flow through the collection, waste tubes, and a single tube elution.

5. **Eluate Tubes**

   Each kit includes 1.5 mL microcentrifuge tubes. These tubes are used to collect the sample eluates with the multi tube elution workflows.

   Important: *It is important to remember to open the cap of the microcentrifuge tube before placing it within the carrier. Failure to do so could cause damage to the robot and loss of the sample eluate. Point the open caps towards the waste bag.*

4.6 Additional Consumables Required:

Associated consumables should be purchased separately by the user. Users must use original parts commercially available by the listed suppliers:

Product Name	Catalog Number	Supplier
1000 µL Conductive Tips (3840 tips)	235904	Hamilton
300 µL Conductive Tips (5760 tips)	235902	Hamilton
Biohazard Waste Bags (25 bags)	199203	Hamilton
USER Enzyme + CutSmart Buffer (250 U)	M5505L	New England Biolabs
BioFluidica LiquidScan Kit Options		
B^3 Blood Collection Kit	3000415	BioFluidica
CTC - Solid Tumor Kit - 8 chips	3000418	BioFluidica
CTC - Solid Tumor Kit - 4 chips	3000414	BioFluidica
Fetal Cell - Prenatal Kit - 8 chips	3000428	BioFluidica
Fetal Cell - Prenatal Kit - 4 Chips	3000424	BioFluidica

5

Operations

5.1 Operation of the Instrument

Users should always practice good laboratory technique when performing any tasks with the LiquidScan® System.

5.2 General Workflow Precaution

- Avoid long pauses when tips are carrying liquid, as liquid may leak from the tip.
- Discard all used tips. Do not reuse tips. Do not attempt to wash or sanitize the tips to reuse them.
- Do not empty the tip waste bag during a run.
- Do not leave tips on the pipetting head for prolonged periods of time, for instance overnight. This may damage the mechanism that holds the tips in place.
- If tips remain on the pipetting head after a run or after aborting a run, please read the Troubleshooting Chapter.

5.3 Address System

The address system assigns a number to specific positions on the Hamilton Deck within the LiquidScan software. Hamilton carriers residing on specific tracks, for example the SMP_CAR_24 located on Track 14, has 24 different positions for plasticware and each is given a specific address. These addresses can serve as either a source/aspirate position or a destination/dispense position.

The reagent plate houses the positions associated with addresses 1-96, and the other carriers on the deck house the positions associated with addresses 97-264. The BioFluidica microfluidic chips are not part of the addressing system. Addresses 1-264 are defined sequentially, meaning the address selected as the source or destination position as well as the addresses for the chips that follow will be used.

For example: if address 1 is selected as a source/destination when running 8 chips, the robot will aspirate/dispense to addresses 1-8, or when running 4 chips, the robot will aspirate/dispense to addresses 1-4. As such, these addresses are used for all multi tube applications.

There are some specific addresses that will always be used regardless of the workflow chosen. These are:

- Waste will always be set at 209.
- Enzyme location will always be set at 305.

Specialty methods can be developed if a user would like to aspirate or dispense from a single source or destination. Contact BioFluidica if custom applications are needed for the laboratory.

Reference the figures below to determine tube positions in the LiquidScan STARlet. Note that single tube sample method and multi tube sample method tube positions are different.

Reagent Plate Addresses

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	5	13	21	29	37	45	53	61	69	77	85	93
C	2	10	18	26	34	42	50	58	66	74	82	90
D	6	14	22	30	38	46	54	62	70	78	86	94
E	3	11	19	27	35	43	51	59	67	75	83	91
F	7	15	23	31	39	47	55	63	71	79	87	95
G	4	12	20	28	36	44	52	60	68	76	84	92
H	8	16	24	32	40	48	56	64	72	80	88	96

Multi-Tube Addresses

Rack Position	Track 14	Track 15	Track 16	Track 17	Track 25	Track 26
Volumes:	1mL - 10mL	1mL - 10mL	0.5mL	1mL	1mL - 10mL	1mL
Rack Position	(15mL Round-Bottom)	(15mL Round-Bottom)	(0.5 mL Tube)	(1.5mL Tube)	(15mL Round-Bottom)	(1.5mL Tube)
1	97	121	145	177	209	233
2	98	122	146	178	210	234
3	99	123	147	179	211	235
4	100	124	148	180	212	236
5	101	125	149	181	213	237
6	102	126	150	182	214	238
7	103	127	151	183	215	239
8	104	128	152	184	216	240
9	105	129	153	185	217	241
10	106	130	154	186	218	242
11	107	131	155	187	219	243
12	108	132	156	188	220	244
13	109	133	157	189	221	245
14	110	134	158	190	222	246
15	111	135	159	191	223	247
16	112	136	160	192	224	248
17	113	137	161	193	225	249
18	114	138	162	194	226	250
19	115	139	163	195	227	251
20	116	140	164	196	228	252
21	117	141	165	197	229	253
22	118	142	166	198	230	254
23	119	143	167	199	231	255
24	120	144	168	200	232	256
25			169	201		257
26			170	202		258
27			171	203		259
28			172	204		260
29			173	205		261
30			174	206		262
31			175	207		263
32			176	208		264

Multi Tube Load

Multi Tube Flowthrough >1mL

Multi Tube Flowthrough <= 1mL

Waste always = 209

Multi Tube Elution

USER always = 305

Single Tube Addresses

SINGLE TUBE	Track 14	Track 15	Track 16	Track 17	Track 25	Track 26
Volumes:	1mL - 10mL	1mL - 10mL	0.5mL	1mL	1mL - 10mL	1mL
Rack Position	(15mL Round-Bottom)	(15mL Round-Bottom)	(0.5 mL Tube)	(1.5mL Tube)	(15mL Round-Bottom)	(1.5mL Tube)
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17	289	297	305		313	
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
32						

Single Tube Load = 289

Single Tube Flowthrough = 297

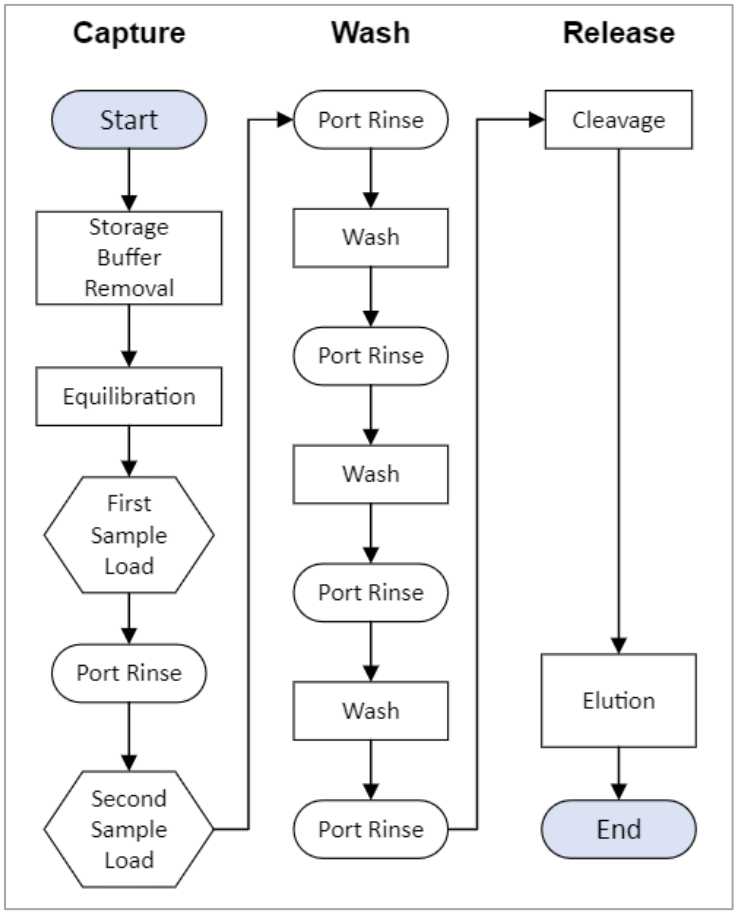
Waste always = 209

Single Tube Elution = 313

USER always = 305

5.4 Sample Enrichment Overview

The workflow for biomarker enrichment is shown in the image below. The process is similar to standard workflows provided by BioFluidica. The three main elements of enrichment are capture, wash, and release of target population.



5.5 Sample Loading Methods

Each chip has a corresponding position for sample load, sample flow-through, and sample eluate. This allows for multiple samples to be processed in the robot without crossover contamination.

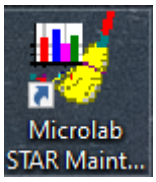
For example, if four different samples are being processed over four chips, the user would place the four samples in the sample carrier positions 1-4. The system will process Sample 1 on Chip 1, Sample 2 on Chip 2, etc.

Specialty methods can be developed if a user would like to aspirate or dispense from a single source or destination. For example, if the user would like to flow a single patient sample over multiple chips, then a custom method can be created. Contact BioFluidica if custom applications are needed for the laboratory.

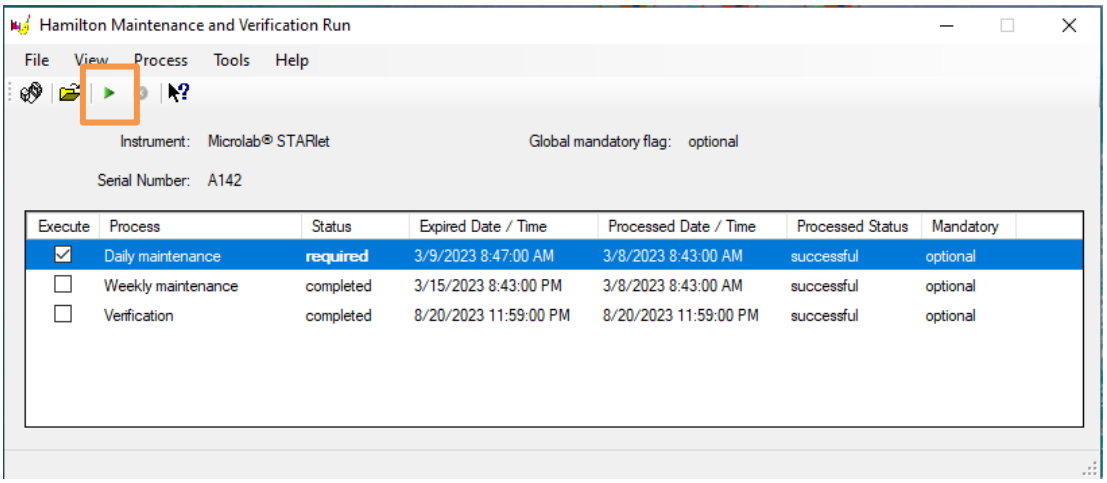
5.6 Setting Up the LiquidScan System

Step 1: Daily or Weekly Maintenance

1. Remove reagent plate and chips from 2-8°C storage and allow 15 minutes to equilibrate to room temperature.
2. Centrifuge the BioFluidica 1mL deep well reagent plate for 1 minute at 1000xg.
3. Turn on LiquidScan STARlet by flipping the green toggle switch on the left side of STARlet upwards.
4. Open the front cover and pull all labware carriers from the Hamilton Deck onto the loading tray. Ensure nothing is in the path of the autoload drive.
5. Execute maintenance and verification by opening "Microlab STAR Maintenance & Verification Software" from Hamilton.



6. Click the check box of the desired maintenance procedure and click the green "Run Process" button next to the red x circle.



7. The Hamilton Run Control window will guide the user through the process of conducting maintenance.

   Important: *If weekly maintenance is processed, then daily maintenance is not required. The software will prompt for specific actions. Follow all onscreen prompts until maintenance is complete. See chapter 6 for more on Maintenance.*

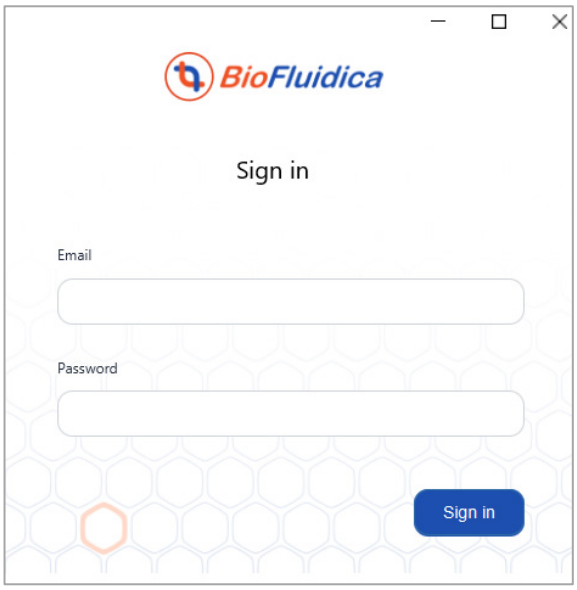
Step 2: Loading the LiquidScan STARlet

1. Ensure proper tips, samples, and tubes are loaded in the appropriate carrier on the loading tray. Appendix A outlines the deck modules and their positions.
2. After loading the proper labware, push in all carriers, racks, and the LiquidScan Module. To push in the components, depress the black labware stop hooks on the furthest right track and push the labware rack all the way to the back.
3. Ensure that the blue running light surrounding the module and the orange BioFluidica logo on the front of the module are lit up. This indicates that power is actively being supplied to the module.
4. Place the reagent plate on a hard surface and carefully remove the foil cover. Place the reagent plate within the carrier so that position A1 is in the upper left-hand corner and the BioFluidica sticker is facing the right side of the instrument.
5. Aliquot samples into provided 17x100mm round bottom tubes (BioFluidica Catalog #: 3000203) and place these aliquots or 10mL blood collection tubes into the carrier on track 14. For example, if there are eight samples, they would be loaded into track 14, positions 1-8. Note that an extra 500µL of sample is required to account for the dead volume of the tube.
6. Place the USER enzyme into the molded tube carrier insert in track 16, position 17. Refer to Appendix D for the USER enzyme recipe. The USER enzyme can be made before a run or made immediately before the USER enzyme step.
7. Place flow-through tubes in track 15, positions 1-8.
8. Place the BioFluidica module on tracks 18-23.
9. Remove the chips from the foil packaging and remove the parafilm wrapping. Chips are labeled with a sticker indicating their ID number. It is helpful to place the chips from lowest number to highest number on the deck from back to front so that the relative position will always be known.
10. Open the orange cover of the BioFluidica LiquidScan Module and secure the microfluidic chips by placing the positioning holes of each chip on the pins of the deck. Place the chips on the left side of the deck for the enzymatic cleavage method.
11. Close the orange cover, depress the labware stop for track 23, and push the carrier all the way to the back of the deck. Ensure that the module is pushed as far back as possible.
12. Place reagent waste tubes on track 25, positions 1-8.
13. Place the eluate tubes on track 26, positions 1-8. Label the tubes properly, and open caps before putting them on the deck.

Step 3: Running LiquidScan Software

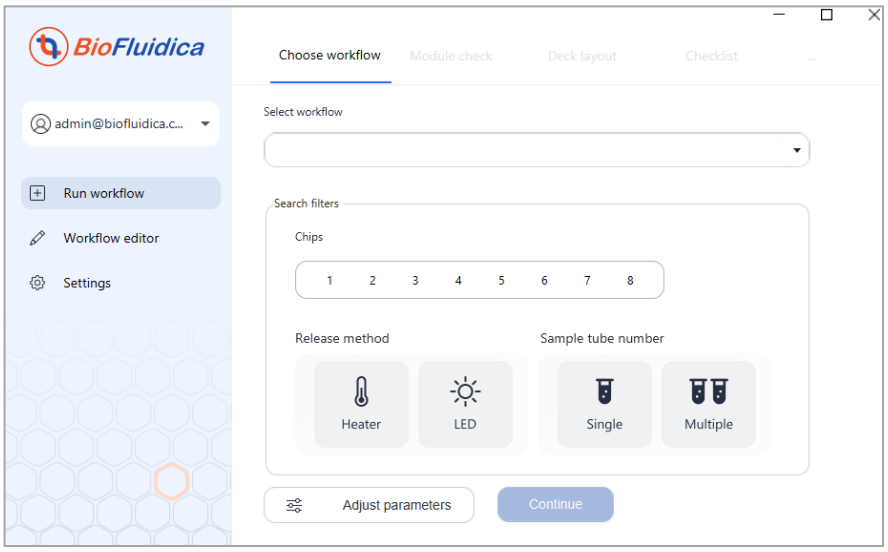
1. **Choose Workflow:**

1.1. Open “LiquidScan Software” and log in.



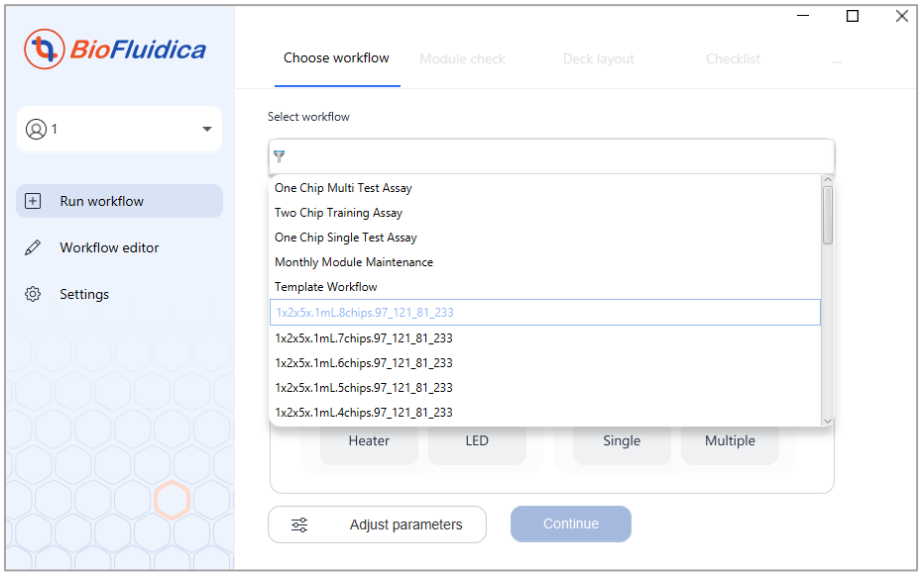
1.2. Contact your lab administrator for additional user accounts.

1.3. Choose the workflow on the “Run Workflow” page.

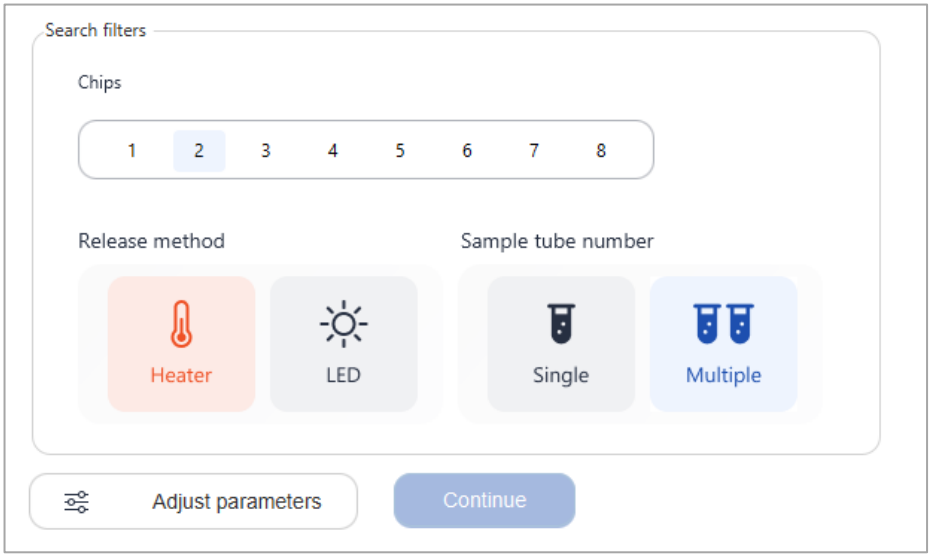


1.4. There are two methods for choosing the workflow.

1.4.1. First method: click on the dropdown menu under “Select Workflow.”



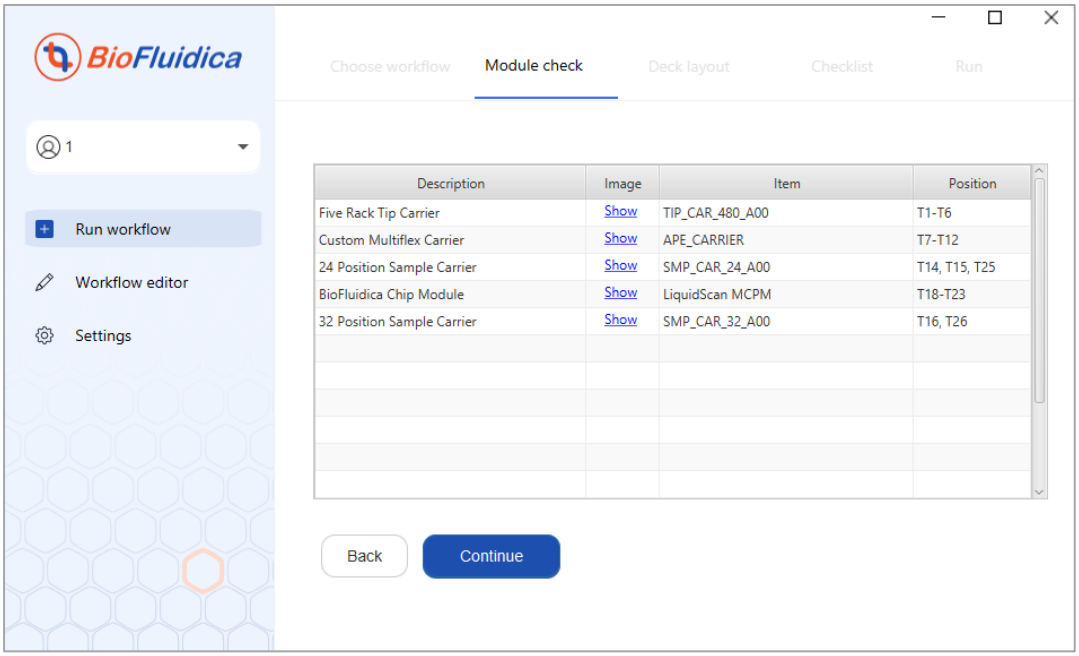
1.4.2. Second method: Use the Search filters by selecting any combination of number of "Chips," the type of "Release Method," or "Sample Tube Number." You can use single or multiple filter options.



1.5. You can reset any selected search filters by clicking on "Run Workflow" in the left navigation bar.

1.6. Once the workflow is selected, click "Continue" found at the bottom of the window.

2. Module Check:



2.1. Please note that by clicking on "**Back**", you will return to the Run Workflow screen.

2.2. The "Module Check" page will have the list of items needed for the assay.

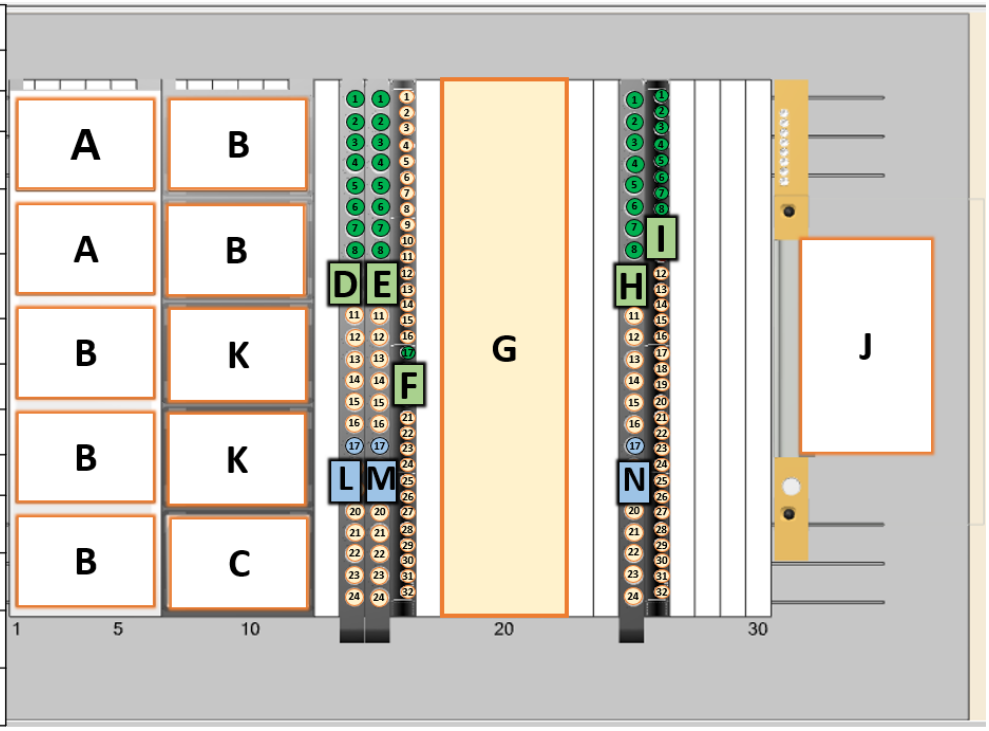
2.3. Verify that the correct component is placed in the correct location of the robot by looking at the "Image", "Item number", and "Position".

2.4. The position will indicate how many rows the component covers on the robot.

2.5. Click "Continue".

3. Deck Layout

A	1000 µL Tips
B	300 µL Tips
C	Plate Holder
D	Multi Load Sample Tubes
E	Flow Through Tubes
F	USER Enzyme Position 17
G	Microfluidic Chips
H	Waste Tubes
I	Elution Tubes
J	Waste Bag
K	Alternate Eluate Positions
L	Single Load Sample Tube
M	Single Tube Flow Through
N	Single Tube Elution



3.1. The “Deck Layout” page will load a diagram of the robot deck map.

3.2. Use the diagram to verify that the components are placed correctly.

3.3. When placing the chips:

3.3.1. Ensure that there is a drop of PBS on each chip port.

3.3.2. Ensure that the chips are pulled towards the bottom right corner on the BioFluidica Module.

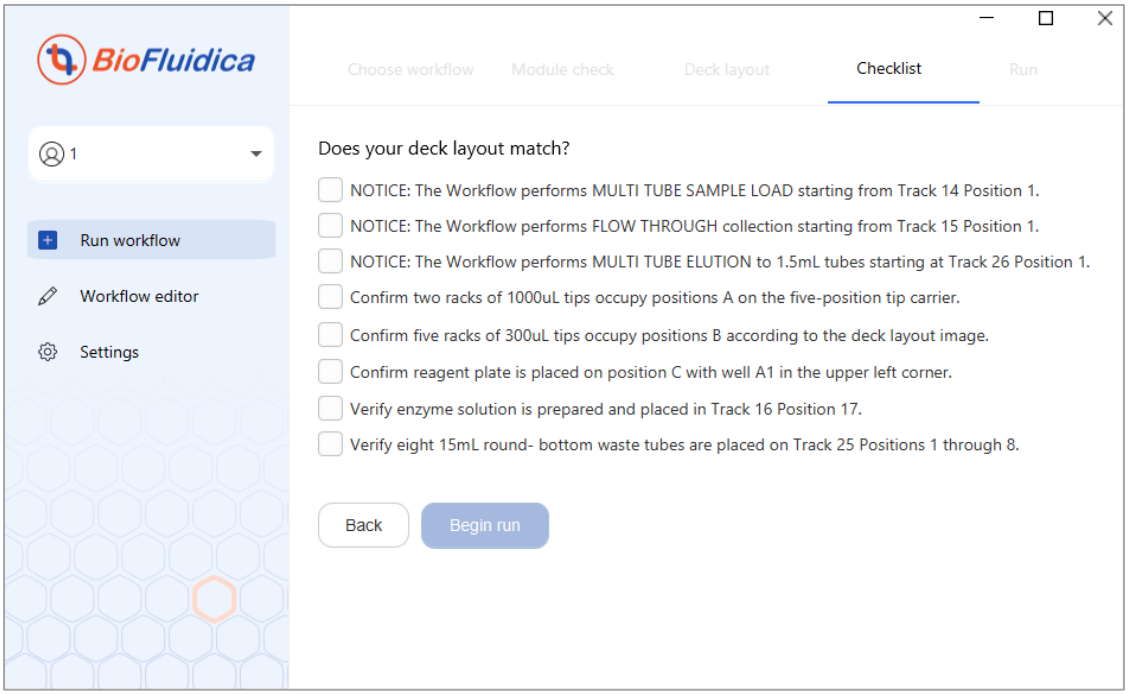
3.4. Ensure that all tubes are pushed all the way down in each carrier. Failure to do so may result in pipetting errors, loss of sample, and damage to the robot.

3.5. Ensure that all carriers and the LiquidScan module are pushed all the way back in the robot. Failure to do so may result in pipetting errors, loss of sample, and damage to the robot.

3.6. Click “Yes” to confirm.

3.7. Click “Continue.”

4. Checklist



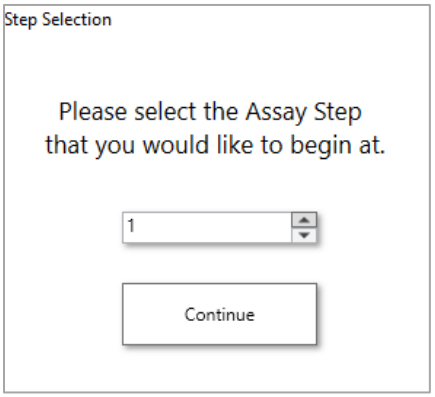
4.1. Read through the deck layout “Checklist”.

4.2. Click on each checkbox after each task is completed. The program will run only after all items are checked.

4.3. Click on “Begin Run.”

4.4. The robot will initialize and begin running the workflow.

5. Step Selection



5.1. Click “Continue” unless the workflow step number needs to be changed.

STEP 4: Collect the Sample Eluates

1. Once the run is complete, eluates from the samples will be ready for downstream analysis.
2. Discard all labware and biohazardous material according to laboratory policies and clean the LiquidScan and Hamilton STAR system according to the Maintenance Chapter.

6

Maintenance

6.1 Repair, Maintenance, and System Servicing

It is the user's responsibility to maintain the instrument on a regular basis. The LiquidScan™ Microlab STARlet will require daily maintenance or weekly maintenance prior to operation.

While Hamilton is responsible for repairing or replacing the Hamilton STARlet components, BioFluidica is responsible for the LiquidScan module and its control components. All items must be decontaminated prior to shipment to BioFluidica.

Important: *Do not attempt to exchange parts or replace any part of the LiquidScan System without consulting BioFluidica.*

6.2 Required Materials for Maintenance

- Disposable gloves
- Personal protective equipment
- Lint free cloth (never use paper products)
- 70% Ethanol
- De-ionized water (to dampen a cloth for cleaning)
- Microlab disinfectant such as Deconex 61 DR or Solarcept (for decontamination cleaning only) – Never use products containing hypochlorite.

Important: *Do not use paper towels or disinfectants containing hypochlorite or bleaching fluids. DO NOT pour liquids onto the deck of the STAR/STARlet instrument. Be sure to clean up any spills immediately to avoid the risk of damaging electrical components that are housed below the deck.*

6.3 Instrument Maintenance

The LiquidScan STARlet requires maintenance and cleaning on a regular basis. The maintenance is performed using the Hamilton application called, "Microlab STAR Maintenance and Verification."

Maintenance is performed according to the following schedule:

- Daily maintenance is recommended before using the instrument each day. Daily maintenance does not need to be run if weekly maintenance has been completed that day.
- Weekly maintenance should be conducted once a week and will take the place of the daily maintenance.
- A six-month preventative service maintenance is suggested by Hamilton and would be performed by a Hamilton field service technician.

6.4 Daily Maintenance

Daily maintenance involves:

- Making sure the deck is clean and clear of any debris.
- Emptying the tip waste.
- Checking the tightness of the 1000 ml channels.
- Verifying cLLD for 1000 ml channels.

When the software prompts to execute daily maintenance for deck and waste, press yes to continue; pressing no will abort the daily maintenance. The instrument will give a series of prompts for the user. The channel tightness and cLLD will be tested using the metal teaching needles in the robot's back right corner, near the tip waste. Once completed, the program will display a message notifying the user that the daily maintenance was successfully completed.

Important: *If maintenance fails, the report can be viewed to ascertain the cause of the failure by going to the File menu and selecting "Open report". Check the Hamilton User Manual for troubleshooting and re-run daily maintenance. If this fails again, contact Hamilton for technical support.*

6.5 Weekly Maintenance

Weekly maintenance is similar to daily maintenance and involves:

- Removing all the carriers from the deck and cleaning them
- Making sure the deck is clean
- Emptying the tip waste
- Checking the tightness of the 1000 ml channels
- Verifying cLLD for 1000 ml channels

After initializing, the program will remove all carriers from the deck using the autoload. Clean all the carriers with mild detergent or 70% alcohol and allow to dry. Check to make sure that none of the carriers are damaged. Remove the tip eject plate and clean/disinfect it with mild detergent or 70% alcohol. The tip eject plate can also be covered with aluminum foil to help reduce contamination. Make sure to rinse well after washing carriers or tip eject plate. The rest of the weekly maintenance will be the same as the daily maintenance.

6.6 Cleaning the Pipetting Channels

To clean a channel, wet a lint free cloth with DI water and clean the outer sleeve (black portion) and stop disk (silver portion).

Important: *Do not get liquid inside the tip channel.*

6.7 LiquidScan Module Maintenance

The LiquidScan Module should be cleaned after every run with 70% ethanol.

Important: *When cleaning the LiquidScan module, only use water or 70% ethanol applied to a damp, lint-free cloth. Excess fluid could seep into the module and damage electrical components that are housed within the module.*

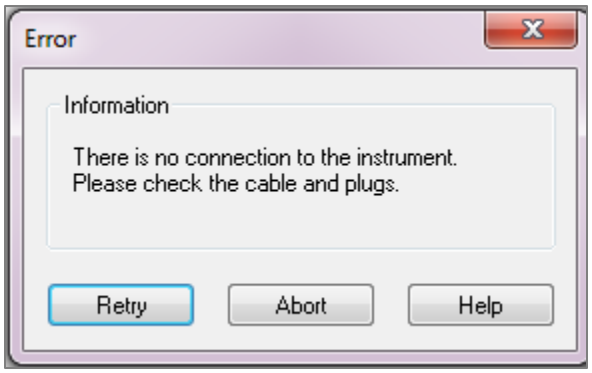
A monthly preventative maintenance should be conducted on the Module to ensure proper function.

a. Open the LiquidScan software and login.
b. On the Run Workflow page under search filters click 8 for the “Chips” selection.
c. Open the select workflow dropdown and select “Monthly Module Maintenance” workflow and click continue.
d. Follow the on-screen prompts and run the workflow.
e. The Software will turn on the heater and initiate a 10-minute pause. Look at the front of the heater and ensure the red number is rising to the set green number below it (37°C). Once the heated side reaches 37°C (about 3 min 30 seconds), open the cover and use gloved hands to feel along the plate to ensure even heating across the plate. The copper plating will be warm, not hot.
f. Close the module cover.
g. Once the heater function has been checked you can proceed to the next step by clicking the “Stop Timers” button.
h. The LED will then start and stay lit for 1 minute.
i. Ensure that none of the LEDS are unlit.
j. If there are any issues with the module contact BioFluidica.

7

Troubleshooting

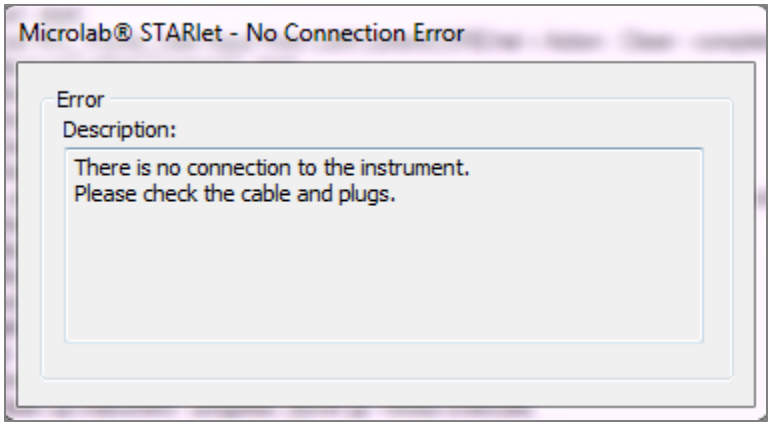
7.1 Instrument Connection Error



One of the most common errors that occur is an Instrument Connection Error. The primary reason this error occurs is because the LiquidScan STARlet is not powered on.

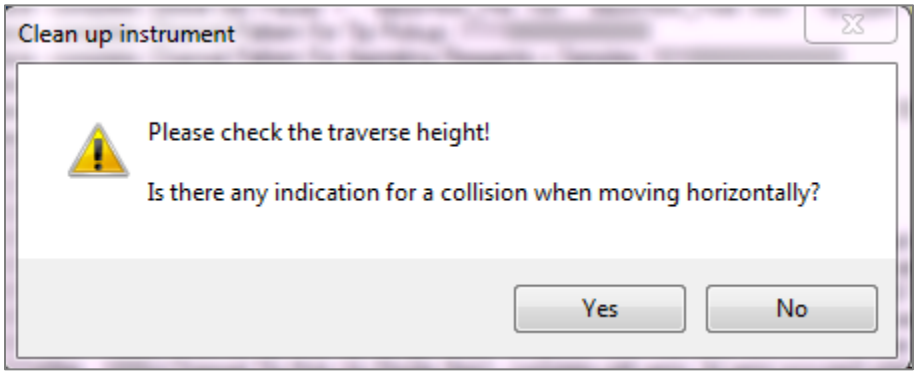
To solve this issue, turn on the LiquidScan STARlet by switching the green toggle switch on the left side up to the (I) position. (The green LED should be on) Click *Retry*, and the method should run.

If the instrument is on and the cables are disconnected, a different pop-up will appear:



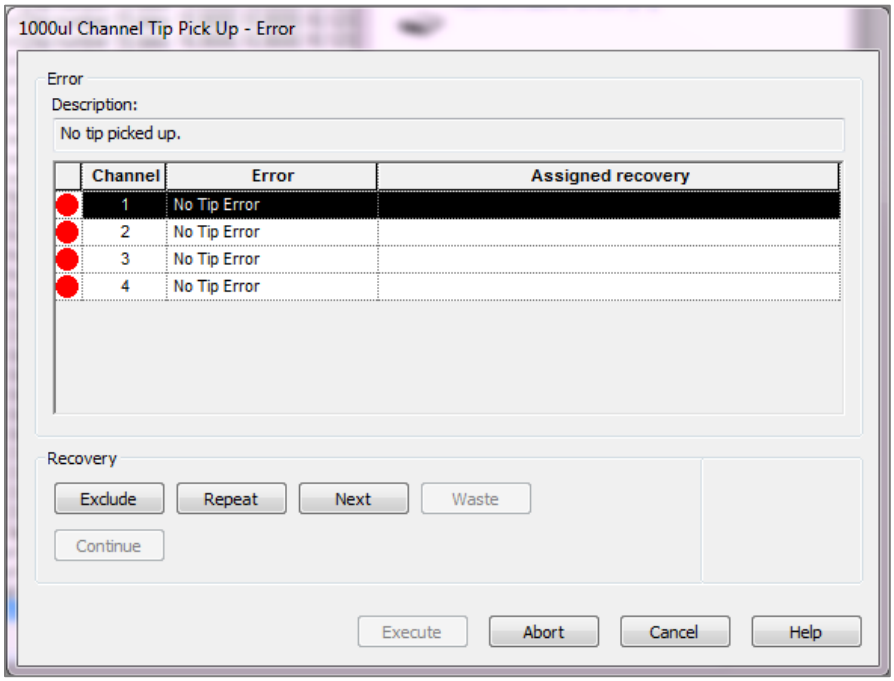
In this case, make sure the cables are connected properly on the LiquidScan STARlet as well as to the back of the desktop. The USB type-B end of the cable should be connected to the instrument in the bottom USB port and the USB type-A end of the cable should be connected to the back of the desktop.

7.2 Tips Remaining on Channels



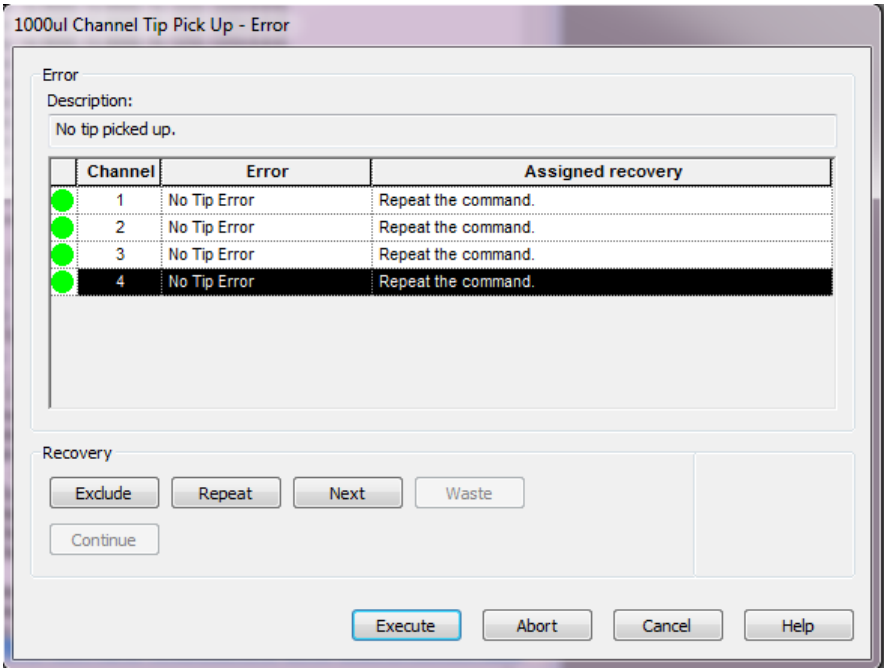
If tips are left on pipetting heads after a run or after aborting a run, running a new method or daily maintenance will remove them. Once the new run begins, the software will ask, “Is any indication for collision when moving horizontally”. Ensure that no collision with either the pipetting arm nor heads or any labware on the deck will occur as the pipetting arm moves horizontally and select “No”. If the user is unable to verify that a collision will not occur, remove all available carriers, racks, and labware from the deck onto the loading tray, then select “No.” Clicking “No” will cause the pop-up to appear a second time. Click “No” again and the instrument will eject the tips into the tip waste bag.

7.3 Failure to Pick Up Tips



If the tips are unavailable or if the tips are missing from specific positions within a rack on the required carrier position, the Hamilton Run Control interface will load the pop-up, “1000 ml Channel Tip Pick Up - Error”. The pop-up will give a description of the error. For example, on the image above, the error

indicates, that there were no tips in the positions from which the channels attempted to pick up tips . The pop-up will indicate which channels were unable to pick up tips with a red circle on the left. When the correct tips are replaced on the carrier rack, click “Repeat”.

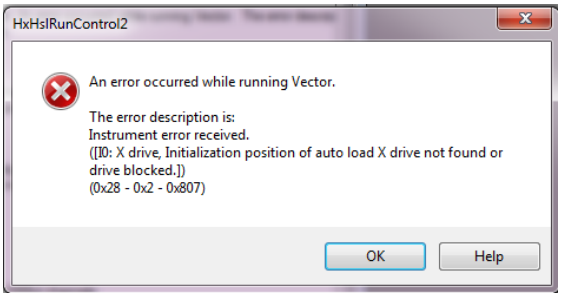


The circles next to the channel numbers will turn from red to green. Clicking “Execute” will tell the system to execute the command and it will pick up the tips from the same position. Alternatively, clicking on “Abort” will abort the run and if any portion number of tips that were already picked up will go to the waste bag.

7.4 An Error Occurred While Running Vector

The software may give errors due to specific circumstances on the instrument. A common error will include a box that states, “An error occurred while running Vector” followed by an error description. Some of the common vector errors are included below.

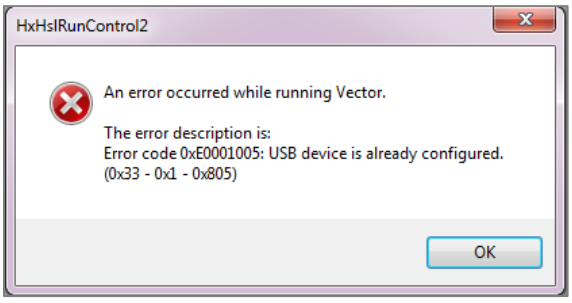
7.4.1 Auto drive error



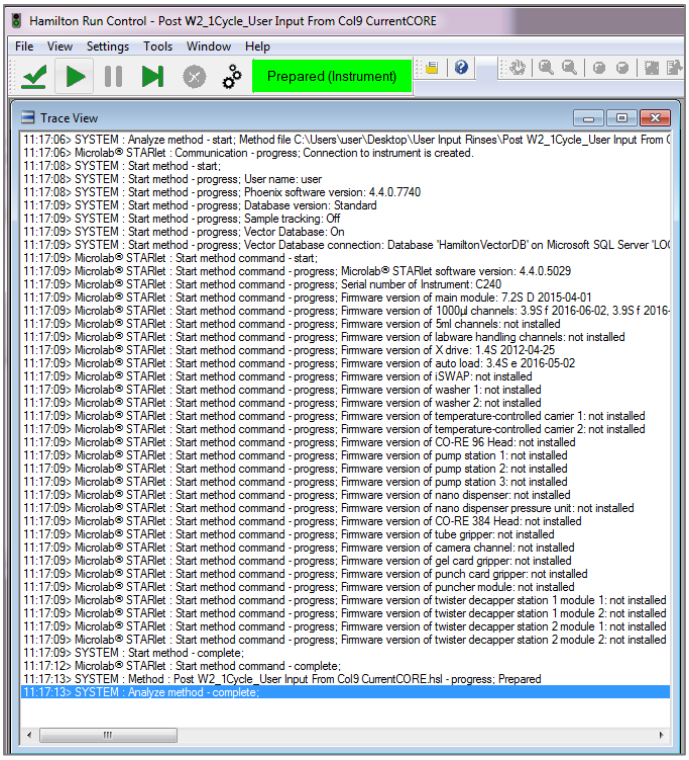
The following is an error in which the autoload drive, which is the red barcode scanner, is blocked typically by a rack or carrier that is not properly positioned on the deck like in the picture below.

To resolve the issue, push the rack or the carrier into the correct position and click ok. The software will abort the current run and the user will be required to restart the run.

7.4.2 Dual run controls



This error occurs when the user attempts to run a method while the Hamilton Run Control is already open. Run Control is one of the three software components and is readily identifiable because it mimics a command prompt interface. Example:



If this window is open, the error message will occur when a new run is initiated. To resolve this issue, close the Hamilton Run Control window and re-run the new method.

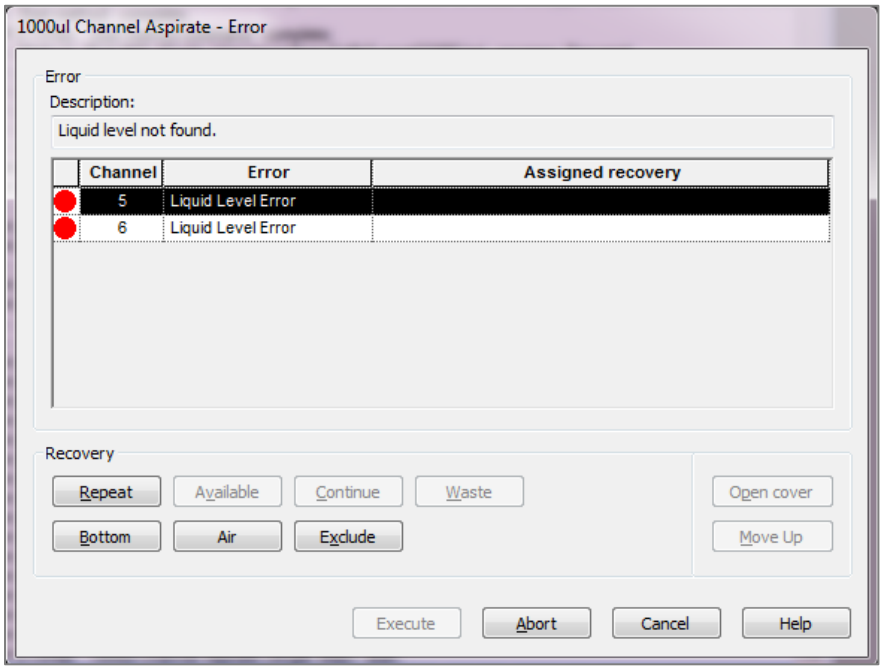
7.5 Robot Pipette Tips Missing Correctly Defined Labware

On the loading deck, there are small black separators to help slide labware into a place known as slide blocks. If system components are improperly placed and do not go between these blocks, it can alter the positioning of labware on the X, Y, and Z planes of the deck. If labware deviates from its defined position in these planes, the robot may malfunction when interacting with the labware. This can lead to other

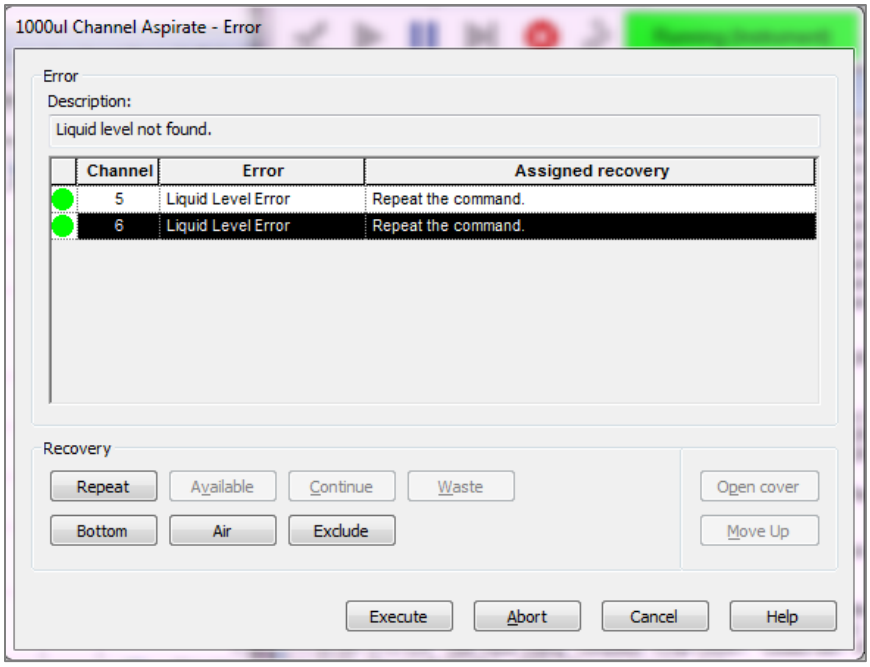
errors. If an error occurs after repeated attempts, check to ensure all components are installed on the deck correctly. In the example shown above, the module is incorrectly positioned on top of the slide blocks instead of between them.

7.6 Liquid Level Errors

The STARlet uses capacitance to detect the liquid level of reagents and samples during aspiration only. If there is insufficient liquid in the position that the robot is attempting to aspirate from the software will give an error.

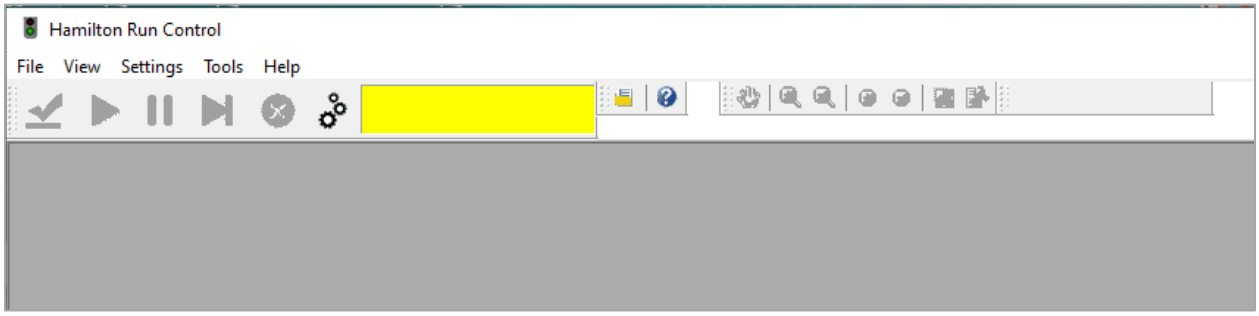


First check that the reagent or sample that the software is attempting to aspirate is in the correct position. If the reagent is missing, for example if the reagent plate has not been place into its carrier position, replace the reagent. This issue can be solved similarly to the insufficient tips error. The software will indicate which channels are unable to aspirate liquid along with the red circle. Clicking on “Repeat” will convert the circles from red to green. Select “Execute” and the channels will repeat the aspiration. Clicking “Abort” will abort the run and move the tips to waste. Another option, that should only be used when you are positive the reagent or sample container contains the correct amount of liquid would be to click on the “bottom” button followed by the “repeat” button. In this case the pipette channel will go to the bottom of the container and aspirate the desired amount of liquid. Use this option with caution and double check the container for liquid level before proceeding with this option. The software does not use any liquid-level detection method while dispensing. The software will dispense reagent or eluate even if the plasticware is not placed into the proper labware location.

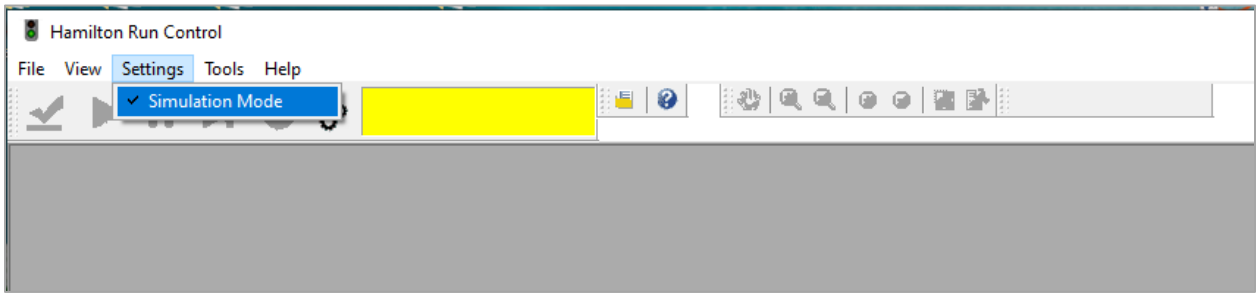


7.7 Simulation Mode

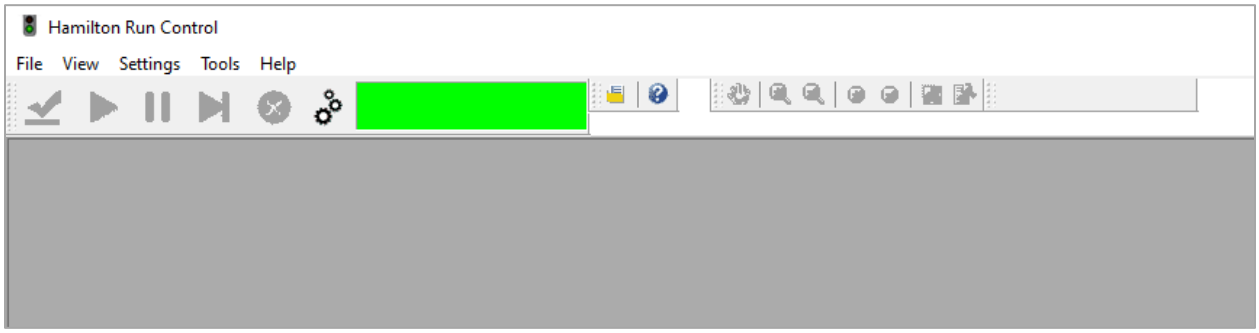
There are times when the system may be in simulation mode. The simulation mode runs a specific method without executing any actions on the robot. This may make it seem to appear that the robot is not actually working. There are a few ways that you can tell that you are in simulation mode. The first is upon opening the run control program the colored box will yellow.



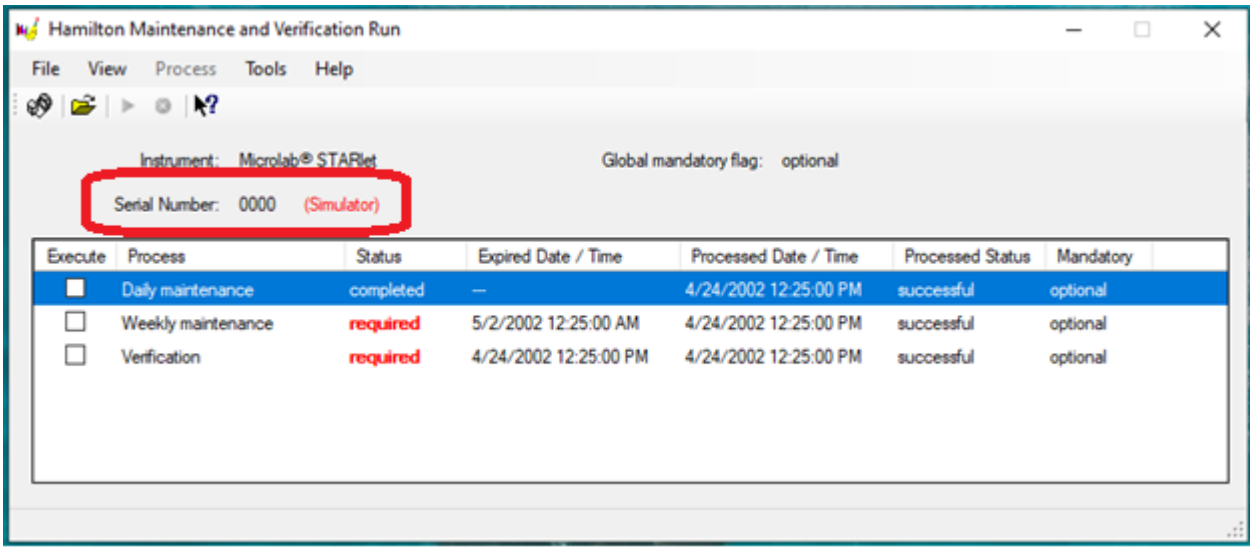
In order to set the system back to the proper run operation, click on settings and uncheck the "Simulation Mode".



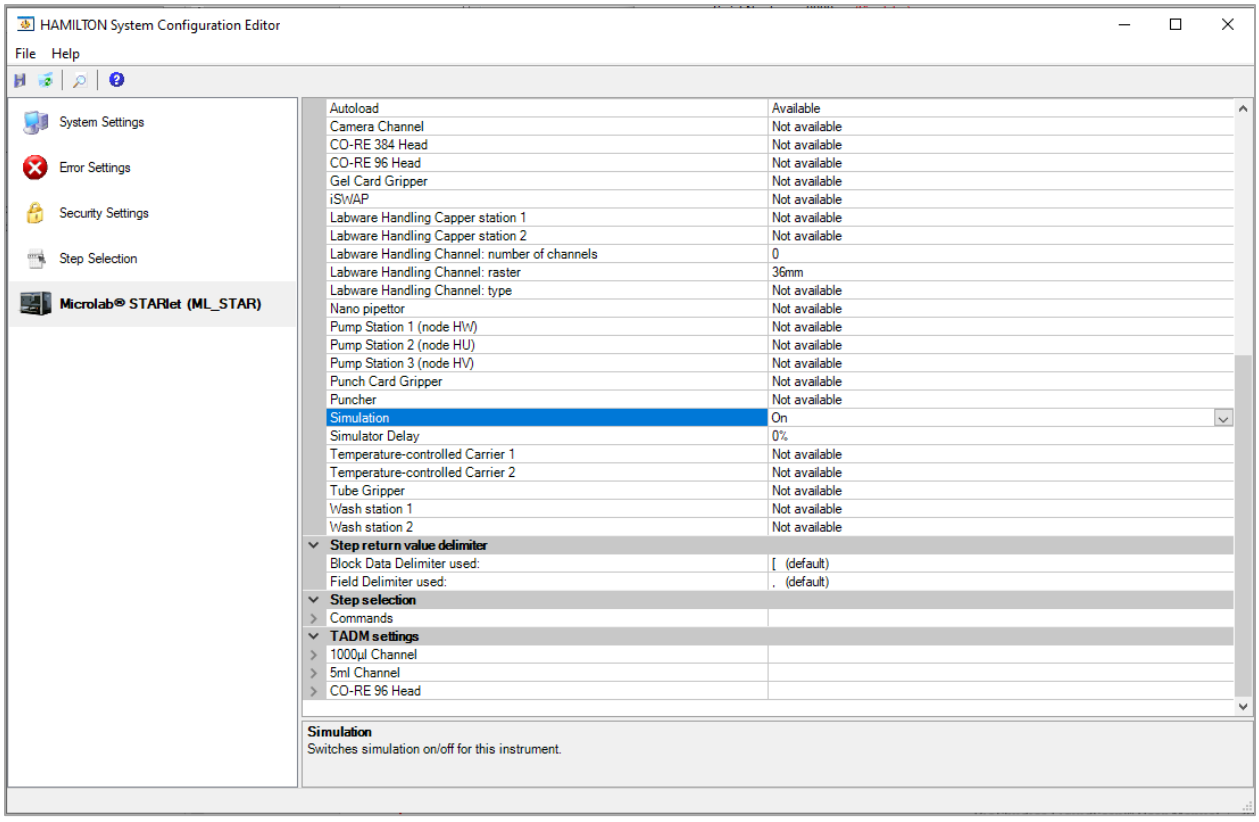
The system should now have a green box instead of yellow, and the system is out of simulation mode.



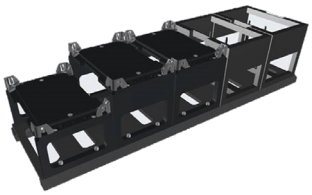
The second method is upon opening the “Hamilton Maintenance and Verification Run” software the system will say “Simulator” in red as shown below. You will be unable to run the required maintenance. You will need to exit this program and follow the instructions above and re-open the Maintenance software and you will then be able to execute the daily or weekly maintenance.



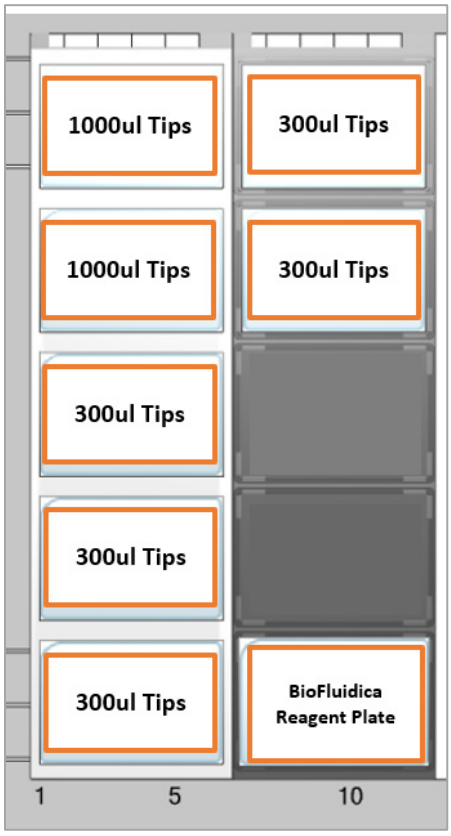
The final method is to open the system configuration manager within any of the Hamilton software, e.g., Hamilton Run or Hamilton Method Editor. Under the Microlab STAR tab, check to see if Simulation mode is on. If it is on, click the drop box, change the setting to off, save, and close the software. The system will now be out of simulation mode. It may be necessary to check the initial step from this troubleshooting section as well as this step because it is possible for the system to be in simulation mode in only the Hamilton Run software while simulation mode is disabled in the system configuration editor.


HAMILTON System Configuration Editor
File
Help
System Settings
Error Settings
Security Settings
Step Selection
Microlab® STARlet (ML_STAR)
Autoload
Available
Camera Channel
Not available
CO-RE 384 Head
Not available
CO-RE 96 Head
Not available
Gel Card Gripper
Not available
iSWAP
Not available
Labware Handling Capper station 1
Not available
Labware Handling Capper station 2
Not available
Labware Handling Channel: number of channels
0
Labware Handling Channel: raster
36mm
Labware Handling Channel: type
Not available
Nano pipettor
Not available
Pump Station 1 (node HW)
Not available
Pump Station 2 (node HU)
Not available
Pump Station 3 (node HV)
Not available
Punch Card Gripper
Not available
Puncher
Not available
Simulation
On
Simulator Delay
0%
Temperature-controlled Carrier 1
Not available
Temperature-controlled Carrier 2
Not available
Tube Gripper
Not available
Wash station 1
Not available
Wash station 2
Not available
Step return value delimiter
Block Data Delimiter used:
[(default)
Field Delimiter used:
, (default)
Step selection
Commands
TADM settings
1000µl Channel
5ml Channel
CO-RE 96 Head
Simulation
Switches simulation on/off for this instrument.

Appendix A - Deck Modules and Position

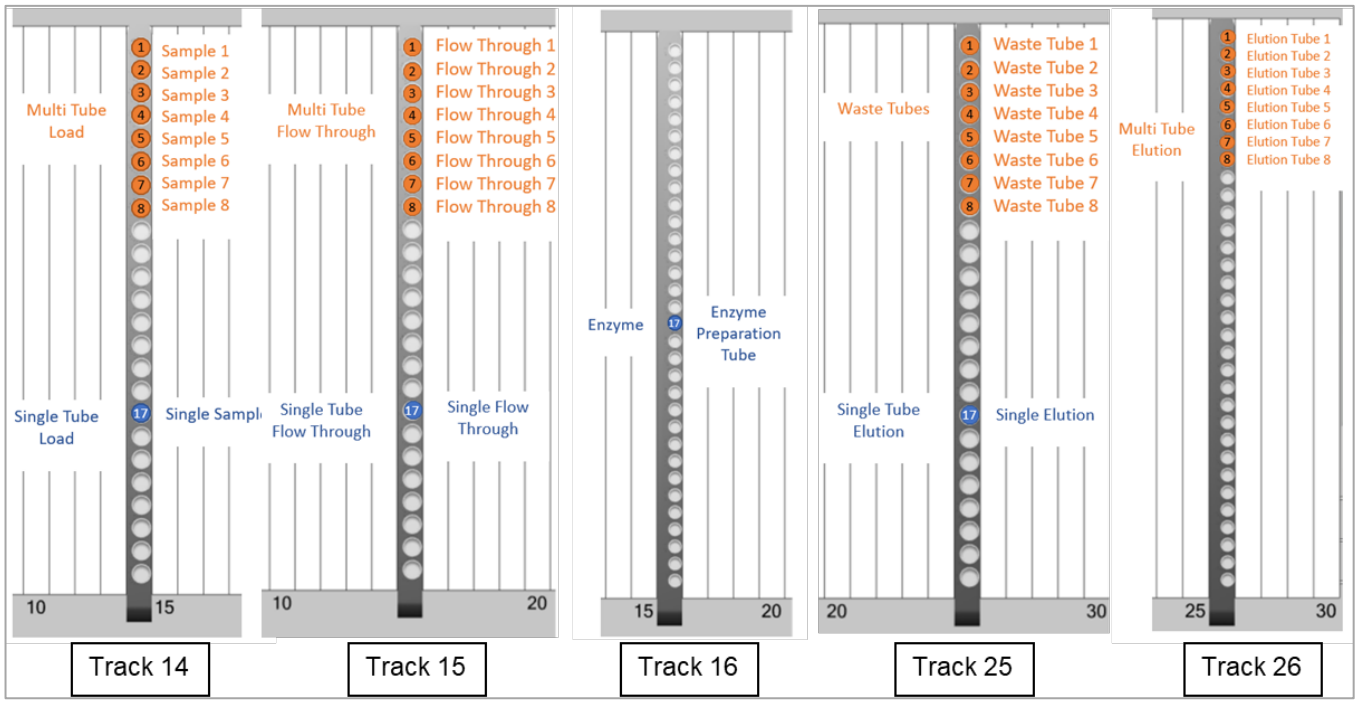
Description	Image	Designation	Position
Five Rack Tip Carrier		Tip_CAR_480_A00	Track 1-6
Custom Multiflex Carrier		APE_CARRIER	Track 7-12
24 Position Carrier for 17 x 100 mm Round Bottom Tubes		SMP_CAR_24	Track 14, Track 15
32 Position Carrier for Enzyme Tube With 1 Microcentrifuge Tube Insert In Position 17		SMP_CAR_32_A00	Track 16
BioFluidica Deck Module		LiquidScan Module	Track 18-23
24 Position Carrier for 15ml Waste Round Bottom Tubes		SMP_CAR_24_A00	Track 25
32 Position Carrier for Elution Tubes With 8 Microcentrifuge Tube Inserts In Positions 1-8		SMP_CAR_32_A00	Track 26

Appendix B - Tip Positions



Description	Rack Position	Tip Type	Designation	Deck Position
Five Rack Tip Carrier	Rack 1	1000 uL	Tip_CAR_480_A00	Track 1-6
Five Rack Tip Carrier	Rack 2	1000 uL	Tip_CAR_480_A00	Track 1-6
Five Rack Tip Carrier	Rack 3	300 uL	Tip_CAR_480_A00	Track 1-6
Five Rack Tip Carrier	Rack 4	300 uL	Tip_CAR_480_A00	Track 1-6
Five Rack Tip Carrier	Rack 5	300 uL	Tip_CAR_480_A00	Track 1-6
Multiflex Carrier	Rack 1	300 uL	APE_CARRIER	Track 7-12
Multiflex Carrier	Rack 2	300 uL	APE_CARRIER	Track 7-12

Appendix C – Tube Positioning on the 24 and 32 Position Sample Carrier



Appendix D - USER Volumes

Chip Count	Nuclease Free Water	CutSmart Buffer	USER	Total
8 Chip	200 uL	40 uL	160 uL	400 uL
4 Chip	110 uL	22 uL	88 uL	220 uL

Appendix E - Product Catalog

Catalog #	Products
2000602	Pillar Microfluidic Chip - Native
2000603	Sinusoidal Microfluidic Chip - Native
3000211	B^3 Blood Collection Tube
3000210	B^3 Blood Collection Tube, pack of 100
3000414	CTC - Solid Tumor Kit - 4 chips
3000418	CTC - Solid Tumor Kit - 8 chips
3000424	Fetal Cell - Prenatal Kit - 4 Chips
3000428	Fetal Cell - Prenatal Kit - 8 Chips
3000818	Reagent Plate Kit - 8 chip
3000201	Equilibration Buffer
3000202	Wash/Elution Buffer
3000203	17 x 100mm Round Bottom Tube (pack of 100)
3000204	1.5mL Elution Tube (pack of 40)
4000587	Ambient Saf-T Shipping Kit
Hamilton Company	
235902	300 uL Conductive Tips (5760 tips)
235904	1000 uL Conductive Tips (3840 tips)
199203	Biohazard waste bags (25 bags)
New England BioLabs (NEB)	
M5505L	USER Enzyme + CutSmart Buffer (250 U)

Glossary

Abort: The command to cancel a specific step or method from completing. The abort process is not immediate. The robot will complete the current step then execute the abort.

Address system: Numbering system that specifies specific labware locations on the Hamilton deck within the Liquidscan software.

Aspiration: The act of drawing up reagent or liquid by the pipette.

Autoload unit: Device enabling automatic loading of carriers on the Microlab STAR/STARlet. It contains a loading head that moves in an X direction, shunts carriers onto and off the Microlab STAR, and reads the barcodes of carriers, tubes, and microtiter plates.

Carrier: A specific type of unit used to hold labware such as tubes, tips or plates.

cLLD: Capacitive Liquid Level Detection is a feature of the Microlab STAR/STARlet used to sense the level and presence of conductive liquids in containers on the deck.

Container: Container is any vessel, for example a tube or plate, that resides in labware on the deck.

Deck: The working area of the robot that is accessible to the pipetting arm for the movement of reagents.

Deck Layout: A deck layout is a defined set of labware that resides in a specific location on the STAR deck.

Dispense: The distribution of a liquid or reagent from the pipette after aspiration.

Hardware error: An error caused by a specific piece of hardware, for example a pipette crashing into a piece of labware.

Labware: Labware is defined as a specific molded plastic or metal carrier created by either Hamilton or BioFluidica that resides on the deck and takes up a specific number of tracks on the deck.

Labware stop: A depressible tab on the first rail just prior to the autoload track that needs to be depressed on the furthest right track of the labware in order to be able to slide the labware onto the deck.

LiquidScan System: The entire processing system used for the capture of rare cells or extracellular vesicles. The system consists of the liquid handling robot, the module, the microfluidic chips, and the LiquidScan kit.

Loading tray: A removeable area at the front of the instrument that allows for loading, unloading, and setup of labware to be used on the deck.

Loading, unloading: The process of placing labware either on or off the deck along the defined track of the deck space.

Method: A program that instructs the instrument how to move reagents or liquids across containers on the labware residing on the deck. A method can be a set of command steps linked together or may itself be a single step within a workflow.

Microfluidic Chip: An injection molded plastic chip with channels or spaces that contain pillars for the capture of rare cells or extracellular vesicles.

Module: Instances of module in this user manual are a specific reference to the LiquidScan module that contains a heating and LED element and functions to keep the microfluidic chips in place for processing.

Pause: A pause in the processing of a step or method. A pause is not instantaneous and will execute after the specific action the instrument is performing at the time, i.e. aspiration or dispense.

Position: A position refers to a specific slot or rack within a piece of Hamilton labware where containers are placed. Positions are numbered from lowest at the rear of the instrument to highest.

Rack: A grouping of containers that resides on a specific piece of labware, for example on the Five position tip carrier each of the 5 slots for tips can be considered either a rack or a position. Rack/postion 1 is located toward the rear of the instrument and rack/position 5 is located nearest to the operator at the front of the instrument.

Track: A track are a specific set of rails that are defined as a track position in which labware resides on the deck. There are 30 tracks on a STARlet and 55 track on a STAR.

Waste: A plastic bag on the right side of the instrument used for collecting tips that have processed liquid or reagent.

Workflow: A workflow is a series of steps or methods in a specific order that define reagent or sample processing across a microfluidic chip that is specific for a desired downstream application.

BioFluidica
3377 Carmel Mountain Road, San Diego, CA. 92121
858-535-6484
info@biofluidica.com
www.biofluidica.com