Quattroport Assay assembly and use guide

Basrur_Vosshall, et al.

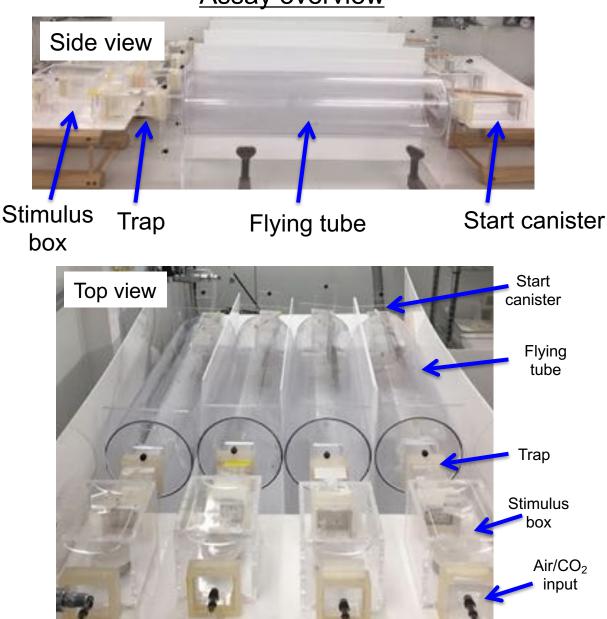
created by: Maria Elena De Obaldia

https://github.com/VosshallLab/Basrur_Vosshall2020

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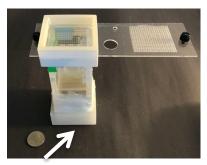
Assay overview



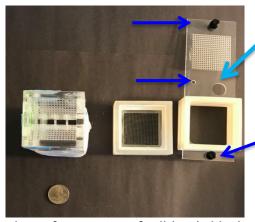
- The Quattroport has 4 "ports", each can be used to test mosquito attraction to a single stimulus. The assay sits on a picnic table (part B005CLO68E),and uses 2 wooden wine racks (part B0000DDUFO, covered with white acrylic part 8505K742) to elevate the start canisters and stimulus boxes 5.75" above the table, so that they can connect with the flying tubes. The stands for the flying tubes sit on the table. The air flow setup sits on the table in front of the stimulus boxes. White acrylic sheets are used to block visual cues, as shown above.
- The Quattroport is modular. It is assembled for one day of use and then disassembled for cleaning at the end of the day.
- All parts are washed with soap and water (or in a dishwasher using baskets part B000096ONX). Once clean, all parts should be handled with gloves.

Parts overview> 3D printed "Stops" and "Sliders"

- Square 3D printed joins are used throughout the assay to connect acrylic and polycarbonate parts. Parafilm and/or white duct tape is placed between 3D joins and other parts, to create a snug fit.
- "Stops" have mesh inside to prevent mosquitoes from going through.
- "Sliders" hold sliding doors which can be opened and closed, to control mosquito movements (these interface with traps and start canisters)



Trap with 3D printed stop and slider on either end



Top view of trap, stop, & slider (with door)

Insert wood dowel here to keep door open

The door has 3 small holes (arrows), that should be threaded (10/32 thread size) to accommodate a small black plastic screw (see parts list).

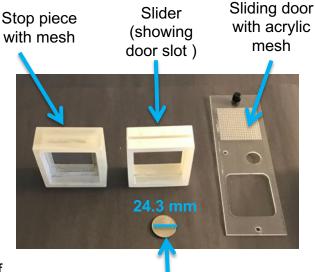


3D printed slider

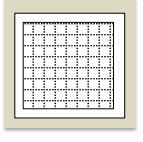
- Need 4+ for trap entrances
- Need 20+ for individual start canisters

3D printed stop with mesh

- Need 4+ for interface b/t trap & stimulus box
- Mesh is sandwiched w. acrylic glue between 2 acrylic "square within a square" pieces, and then super-glued to the side of the 3D printed Stop piece with less "bite"/depth.



U.S. Quarter for size reference



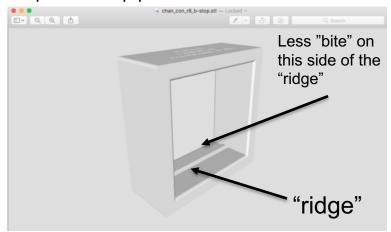
3D printed stop w. acrylic

- Need 4 to connect stimulus box to air supply tubes
- 1/4" acrylic square is connected to the Stop with epoxy, attach acrylic on the side of the piece that has less "bite"/depth.
- Hole is threaded (1/8 NPT) and Female Quick Turn Coupling (Part 9198T17) is inserted here to attach Flexible Tubing "A" (3/8" ID) using the corresponding male Quick Turn Coupling (part 9198T16)

Parts overview> 3D printed "Stops" and "Sliders"

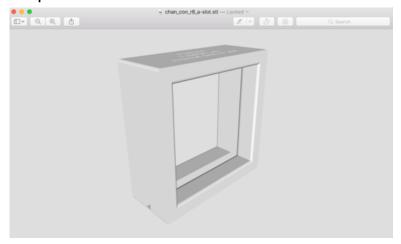
(2 files)

3D printed stop piece



chan con r8 b-stop.stl

3D printed slider



chan con r8 a-slot slider.stl

Notes:

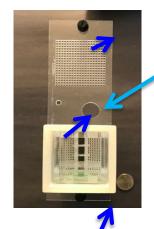
- In the middle of the stop piece, there is a raised ridge, which is used to attach acrylic parts to the 3D printed piece.
- The stop piece is asymmetrical around this ridge. There is more "bite"/depth on one side of the ridge than the other side.
- The side with more "bite" is used to attach the stop to hard polycarbonate tubing, such as on the stimulus box.
- Thus, it is important to glue acrylic parts to the the side of the Stop piece that has less "bite".

Parts overview > Trap and sliding door

- Traps are made from laser cut acrylic (1/4", 1/8", and 1/16")
- The trap has 4 interlocking walls, plus some smaller pieces that make the entrance of the trap (see below)

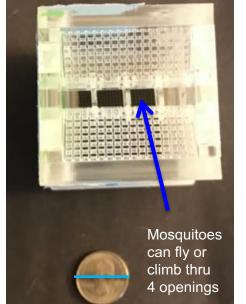


Trap (side view) with sliding door open (mesh above trap)



Insert wood dowel here to keep door open, remove dowel to close the trap

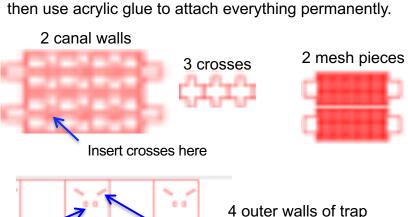
Screws keep the door inside the slider, and the middle screw can be used to keep the door shut in the closed position for transport of live mosquitoes in the trap or start canister.



Trap entrance (top view)
US Quarter for size reference

24.3 mm

- The trap entrance is delicate, assemble it first:
 - Connect 2 canal walls using 3 cross pieces (see below)., it looks like a ladder when assembled.
 - Use acrylic glue to secure & let dry.
 - Insert the 2 prongs of the "ladder" into the 2 small parallel holes on the side of the 1st outer trap wall.
 - Then insert 2 mesh pieces into angled slots on the trap wall, creating the "funnel" part of the entrance
- Then connect the other 3 outer walls of the trap. It takes some finesse to get the trap entrance pieces to connect to trap walls on both sides at the same time.
- Once you achieve this, secure the outer walls with tape and then use acrylic glue to attach everything permanently.



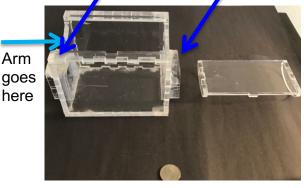
Insert 2 prongs of "ladder"here in parallel slots (interlocking)

Insert prongs of mesh pieces in angled slots

Parts overview > Stimulus box

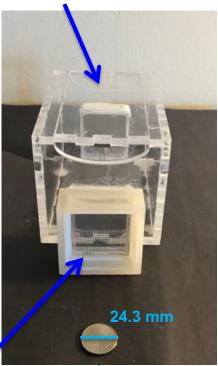
- Stimulus boxes are made of ¼" thick clear acrylic plus two 1.5" pieces of hard polycarbonate tubing (part 3161T41), which should be cut with a band saw and sanded with the deburring tool, then glued with acrylic glue to either end of the stimulus box. This hard tubing interfaces with 3D printed stop/slider pieces.
- When open, the stimulus boxes are designed to hold a human forearm
- Other stimuli can be placed in the box and covered by the lid

hard polycarbonate tube (1.5" piece)



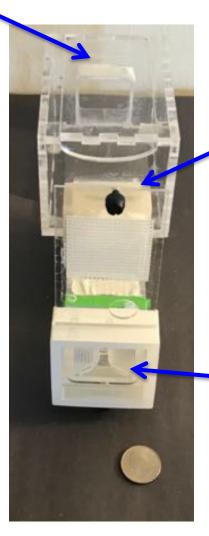
Stimulus box (side view) with cradle to hold human forearm (and lid for when arm is not inserted

Air/CO₂ input this end (3D printed stop piece for air input not shown)



Stop with mesh at this end before trap

U.S. Quarter for size reference

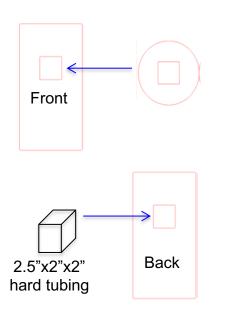


Stop with mesh connects trap & stimulus box

Slider + door at the trap entrance

Parts overview > Support stands for flying tubes

- A total of 8 support stands are needed (2 for each of 4 flying tubes)
- Each stand is made of 2 pieces of ¼" laser cut acrylic (see below) and 1 short piece of hard tubing, which interfaces with 3D printed sliders
- This hard tubing on the support stand connects to the slider on the start canister, providing a conduit for mosquitoes to enter the flying tube.
- Another slider connects the trap to the hard tubing on the stand, providing a conduit for mosquitoes to exit the flying tube and enter the trap.







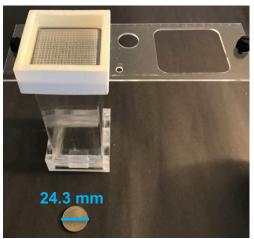


- The 2 pieces of acrylic (rectangle and circle) should be taped together so that the square holes align perfectly. Don't glue it yet, because it matters which side of the circle is glued down (see pro tip below).
- Then, cut a 2.5" piece of 2x2" rectangular hard tubing (part 3161T41) with a band saw. Use deburring tool to file its edges & insert it into the hole on the opposite side of the rectangular piece, (i.e. not same side as the circular piece – see photo). Secure w. acrylic glue.
 - Pro tip: the two sides of any laser cut acrylic will be slightly different, because the side that faces the laser melts more than the other side. So, one side of the laser-cut circular piece will fit snugly into the 5.75" ID polycarbonate flying tube (part 8585K58), but the other may not. Check that the side of the circle you have exposed fits into the flying tube before gluing it down. The flying tube edges may also need to be sanded with the deburring tool, before the circle part of the stand will fit inside it.
- A rubber mallet/hammer is used to attach/detach the flying tubes to/from the stands during routine assembly and disassembly of the assay

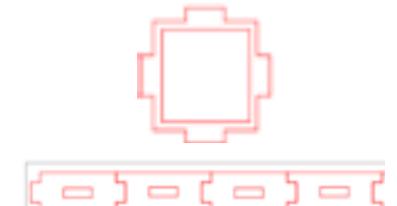
Parts overview > Start canisters

- Mosquitoes are loaded into the start canister the day before the assay is run, so you will need 1 start canister and 1 slider top for each trial you plan to run.
- The start canister is made from a 5.5" piece of 2"x2" square impactresistant polycarbonate square tubing (Part 3161T41). This hard tubing needs to be cut with a band saw.
- The stop piece at the bottom of the start canister is made from interlocking pieces of laser cut acrylic (see below),
- The sliders used in the start canisters are the same as the sliders that interface with the traps.

Start canister with slider (top) and acrylic stop (bottom)

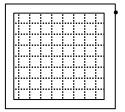


US Quarter for size reference



Laser cut acrylic stop parts

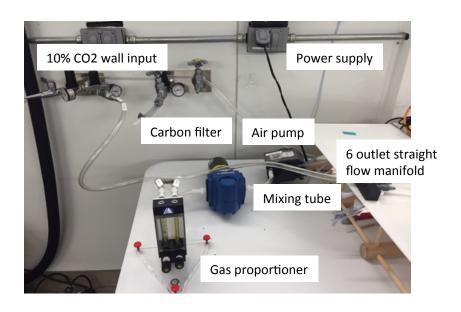
These 4 interlocking pieces have a hole in the center, which is used to snap them onto the acrylic stop square above.



Mesh is sandwiched w. acrylic glue between 2 acrylic "square within a square" pieces (1/16" acrylic), and then attached to the acrylic stop piece with acrylic glue

Quattroport Airflow Explainer - Overview

- The Quattroport uses carbon-filtered humidified room air (RH=80%) mixed with 10% CO₂ (Praxair).
- A flow meter (or gas-proportioner) with 2 flowtubes is used to control the rate of CO₂-containing air into the assay.
- A manifold splits a single source of CO2-containing air into the 4 assay ports.
- Besides this common input source, the assay ports are independent of each other. Each of 4 air streams passes over 4 different stimuli in the stimulus boxes, and then is conveyed through the trap into the flying tube where the mosquitoes are.







Pressure									
MODEL	MOTOR CAPACITY		FLOW V'min illi mbar					MAXIMUM PRESSURE mbar	
	VOLTAGE	TYPE	0	100	200	300	400	CONTINUOUS	INTERMITTENT
60155E (150098)	115/60/1	LM	31.1	10.5	-	-		140	140
6025SE (150057)	115/60/1	LM	58.0	44.6	26.9	10.1	-	280	280
6025SE (150058)	230/50/60/1	LM	60.3	46.5	30.8	8.4		280	280
6025SE (150108)	12	LM	42.5	28.0	12.5	2.1		280	280
6025SE (150109)	24	LM	42.5	28.0	12.5	2.1		280	280

*LM = Linear Magnetic, 230/50/60/1 Model Flow reported at 60 Hz Note: Minimum release quantities or regional availability may apply. Consult factory

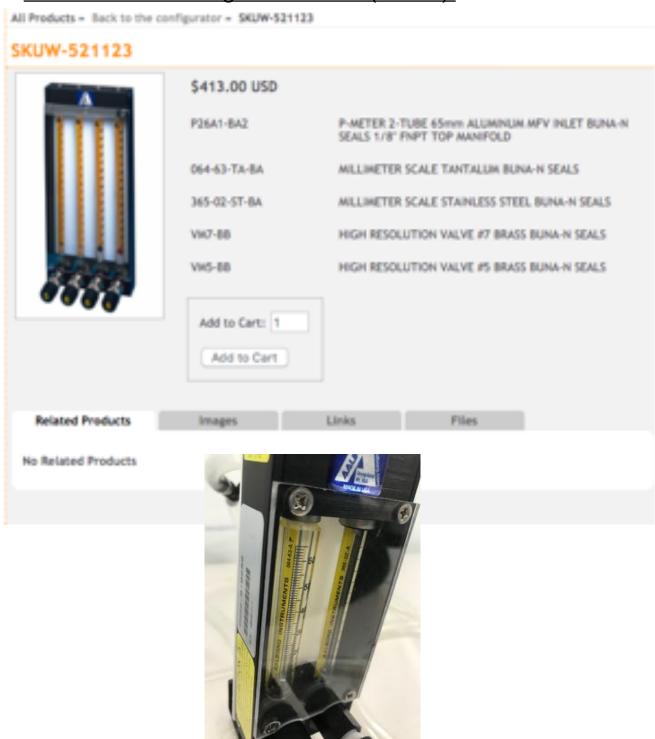
To deliver air and CO₂ into the Quattroport assay consistently, you will need to buy a set of 2 flow tube meters (also called Rotameters or Variable Area Flow meters) from Aalborg. A floating metal ball or "float" is pushed upward, indicating the amount of flow. One needs to consider the material the float is made from to get accurate readings.

Flowmeter Ordering Information:

- Go to Aalborg.com, Select Products>Product Configurator> Rotameters>Model P
- Choose settings and Tube sizes shown below



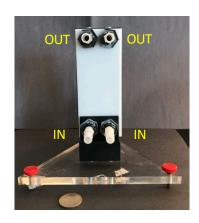
Flowmeter Ordering Information (cont'd):



Quattroport - Airflow Explainer Setup instructions

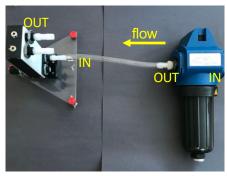
1. Setup flowmeter:

- Attach flowmeter (part P26A1-BA2) to tripod stand (part TP2). Note that each individual glass flow tube is labeled w. its part number.
- b) Screw 4 "K25" male tube fittings (part 2808K25) into the inlets (bottom) and outlets (top) of the flow meter. You will attach 4 pieces of "T16" tubing (part 6546T16) to these fittings in the next three steps below.



2. Connect blue filter housing to the flowmeter, and gray air pump to the blue filter housing:

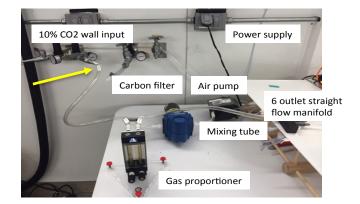
- a) Insert the Carbon filter into the blue Donaldson air filter housing, according to instructions included with filter (not shown).
- b) Attach 2 "K28" male fittings to either side of the blue Donaldson air filter housing. (An arrow on the blue filter housing indicates direction that air should flow through the filter.)
- c) Attach a "K25" male tube fitting (part 2808K25) into the outlet of the Thomas Air pump (labeled OUT). Make sure the inlet of the Thomas Air pump is not blocked during assay operation. Note: Some assembly/soldering is required to connect the Grainger power source to the Thomas air pump please consult the suppliers, Thomas/Grainger, for more info if needed.
- d) Attach approx. 9" of "T16" tubing between the K28 fitting on the outlet of the blue filter housing and the K25 fitting in the inlet(bottom) of the flowmeter that supplies the "065" flowtube for AIR).
- e) Attach approx. 5" of "T16" tubing between the K28 fitting in the inlet of the blue filter housing and the "K25" fitting in the outlet of the Thomas air pump







3. Connect 10% CO₂ source to flowmeter: Attach approx. 2.5 feet of tubing between the fitting in the inlet (bottom) of the flowmeter th supplies the "365" CO₂ flowtube and the 10% tank source (a wall spigot in the photo at right



Quattroport - Airflow Explainer Setup instructions (cont'd)

4. Connect flowmeter to manifold with "mixing tube":

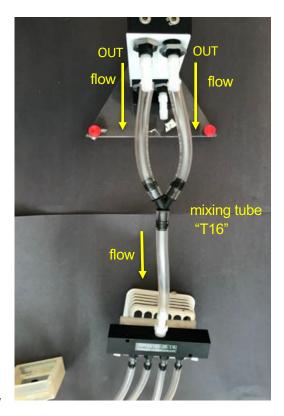
- a) Attach two 5" pieces of "T16" tubing to the K25 fittings at the top of the flowmeter (outlets).
- b) Then connect these two pieces of tubing to the Wye-tube fitting (part 5463K725), as shown here.
- c) Attach a 6" piece of "T16" tubing to the remaining spot on the Wye-tube fitting, this is "the mixing tube".
- d) Attach a "K25" male tube fitting to the inlet of the 6 outlet manifold (part 1023N14), and then connect this fitting to the "mixing tube."

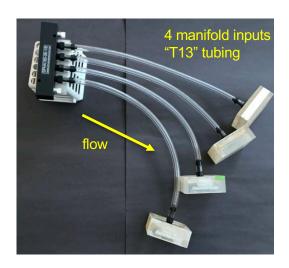
5. Setup the manifold and air/CO₂ inputs:

- Screw silver square plug "K382" fittings into the outer 2 outlets of the manifold (these will not be used.)
- Screw 4 "K24" fittings (part 2808K24) to the middle 4 outlets of the manifold. They will not screw in all the way.
- c) Note: In the final setup, the manifold sits on a 2.5" high piece of plastic on the table and the 3D printed stops with tubing extend up onto the platform on the wine rack where the stimulus boxes are.

6. Prepare n=4 3D printed Stop pieces with air inputs:

- a) Laser cut 4 "stop with air input" parts from ¼" thick clear acrylic. (See "Guide to 3D printed Stops and Sliders" in this PDF.)
- b) Then thread the hole in the center of this part using an 1/8 NPT tap (part 2525A169).
- c) Epoxy the laser cut part inside the 3D printed Stop pieces, allow to dry overnight.
- d) Then screw a "K24" fitting into each acrylic square.
- e) Attach 4 pieces of "T13" tubing (part 6546T13; length =13" each) on the manifold between the K24 fittings on the manifold and the K24 fittings on the stop pieces.
- f) Note: in the final setup, these Stop pieces with air inputs will attach to the back end of the 4 stimulus boxes.





Quattroport - Airflow Explainer Fittings guide

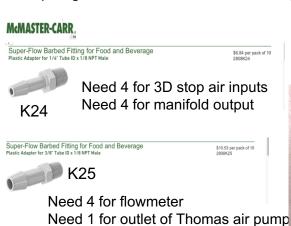
Full product specs uploaded to: https://github.com/VosshallLab/Basrur Vosshall2020

1/8 NPT

K24

1/8 NPT

K25



1/4" tube ID 3/8" tube ID 3/8" tube ID 3/8" tube ID 24.3 mm

K28

1/4 NPT

3/8 NPT

K32

Super-Flow Barbed Fitting for Food and Beverage
Plastic Adapter for 3/8" Tube ID x 1/4 NPT Male

Shape
Straight
Type
Adapter

K28

Need 1 for manifold input



Need 2 for Blue air filter housing



Operation instructions: The standard airflow conditions for the Quattroport are as follows:

- Before beginning, make sure the tripod holding the flowtubes is level.
- Before starting experimentation, adjust the pressure gauge of 10% CO₂ coming out of the wall source, and the CO₂ flowmeter knob (the one with tube number "365"), such that when the CO₂ spigot is turned on, the floating ball reaches the level of "30".
- Mosquito acclimation period (10 min): Air ON ("064" flowmeter reads "15"), CO₂ off (turn off at wall spigot., "365" flowmeter reads "0")
- Assay start (1st 30 sec): Air ON ("064" flowmeter reads "15"), CO₂ stays OFF, stimuli are placed in stimulus boxes during the first 30 seconds of the assay
- Assay duration: Air ON ("064" flowmeter reads "15"), CO2 ON ("365" flowmeter adjusted to reach "30" when CO2 spigot on the wall is turned on

Flow rate calculation guide:

- The standard operating settings for the Quattroport are flowmeter readings of "15" for AIR, "30" for CO₂. These settings provide a total flow rate of 17,393 ml/min of 1.1% CO₂ or ~10,392 ppm above ambient conditions. (See yellow highlighted conditions in table below.)
- Flow rate specs for these flow tubes (with the specified float materials) are reproduced on the following 2 pages from Aalborg's website.
- The table below shows how to calculate the amount of CO₂ delivered using this combination of flowmeter tubes and a 10% CO₂ tank. Using a constant air reading of "15" the % CO₂ delivered can range from 0.05% to 4% (868-23,254ppm), depending on the CO₂ reading used.

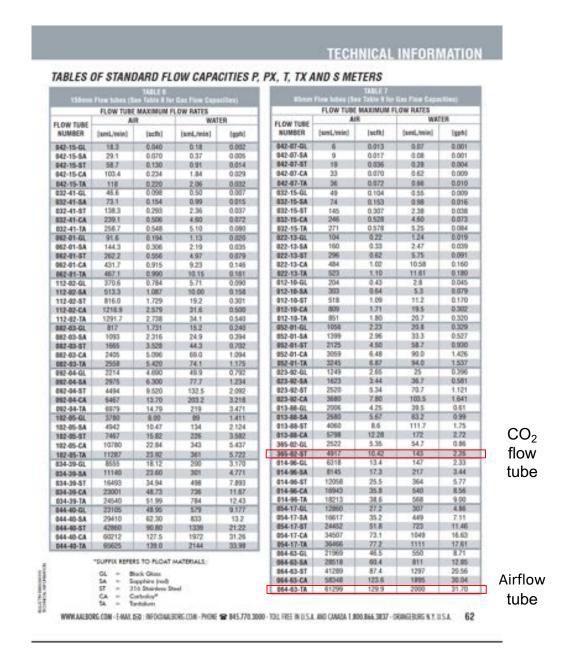
Row	Constant air flowmeter reading (mm)	Air flowmeter 064-63-TA flow rate (ml/min)	Reading (mm)	CO2 flowmeter 365-02 ST flow rate (ml/min)	Total Flow (ml/min)	Fraction of total input from 10% CO2 tank	Final [CO2] fractio n	Final % CO2	ppm CO2	ppm CO2 above ambient
1	"15"	15422	"5"	284	15706	0.018	0.002	0.18%	1808	868
2	"15"	15422	"10"	572	15994	0.036	0.004	0.36%	3576	2636
3	"15"	15422	"15"	952	16374	0.058	0.006	0.58%	5814	4874
4	"15"	15422	"20"	1304	16726	0.078	0.008	0.78%	7796	6856
5	"15"	15422	"25"	1645	17067	0.096	0.010	0.96%	9638	8698
6	"15"	15422	"30"	1971	17393	0.113	0.011	1.13%	11332	10392
7	"15"	15422	"35"	2311	17733	0.130	0.013	1.30%	13032	12092
8	"15"	15422	"40"	2657	18079	0.147	0.015	1.47%	14697	13757
9	"15"	15422	"45"	3043	18465	0.165	0.016	1.65%	16480	15540
10	"15"	15422	"50"	3475	18897	0.184	0.018	1.84%	18389	17449
11	"15"	15422	"55"	3947	19369	0.204	0.020	2.04%	20378	19438
12	"15"	15422	"60"	4373	19795	0.221	0.022	2.21%	22091	21151
13	"15"	15422	"65"	4922	20344	0.242	0.024	2.42%	24194	23254

Table adapted from Aalborg product specs

See these Product sheet PDFs for more info (on Github):

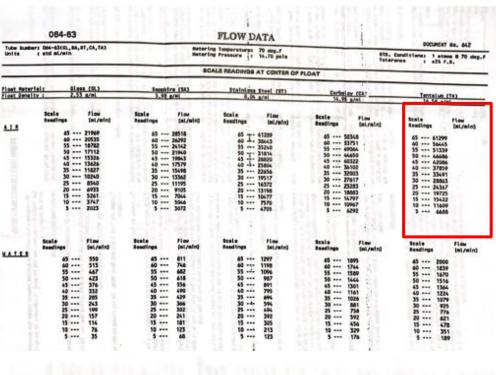
- Aalborg 064-63-TA air tube flow specs.pdf
- Aalborg 365-02-ST CO2 flow specs.pdf
- Aalborg Flow meter instructions.pdf

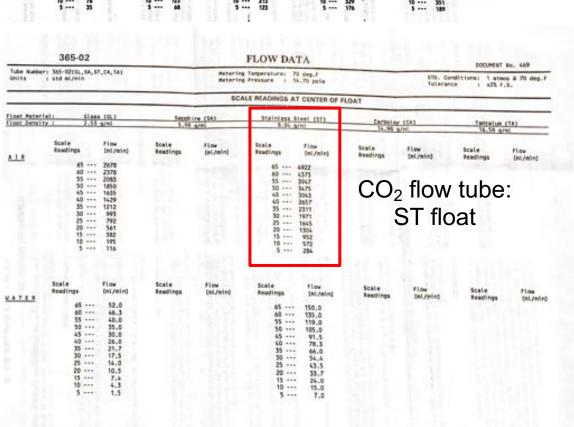
Higher resolution PDF included on Github, filename: Flowmeter en Aalborg EM20200310 Technical Information.pdf



From: Aalborg website

Higher resolution PDFs (on Github): Aalborg 064-63-TA air tube flow specs.pdf Aalborg 365-02-ST CO2 flow specs.pdf





Airflow

tube:

TA

float

Laser cutting & 3D printing guide > Overview

All files needed are found here:

https://github.com/VosshallLab/Basrur_Vosshall2020

<u>Laser cutting files in 3 groups)</u>

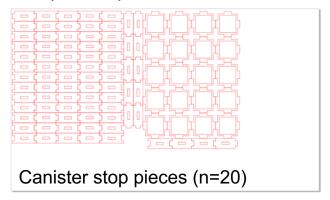
- ¼" (6.35mm) clear acrylic sheets:
 - Flow tube stands (need 4 of these)
 - Stimulus boxes, with lids (Need 4+ of these)
 - Canister stop pieces (need 20+ of these)
 - Attraction trap walls (Need 4+ of these)
- 1/8" (3.175 mm) clear acrylic sheets:
 - Sliding doors
 - Attraction trap "funnel" parts
- 1/16" (1.5875 mm) clear acrylic sheets
 - Square within a square pieces (for traps)
 - Mesh pieces and crosses (for traps)

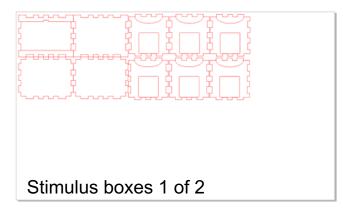
3D printing files:

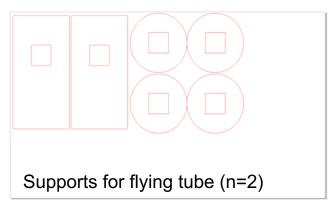
- Stop piece
- Slider piece

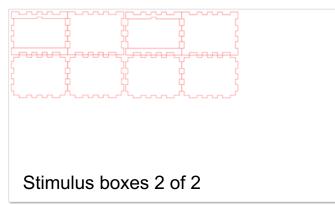
Laser cutting guide >1/4" Acrylic

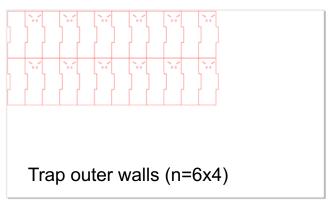
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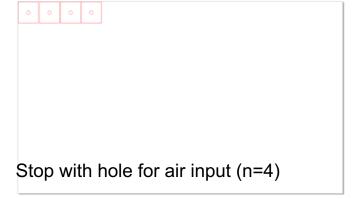






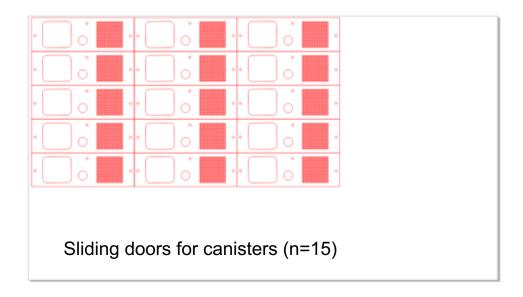


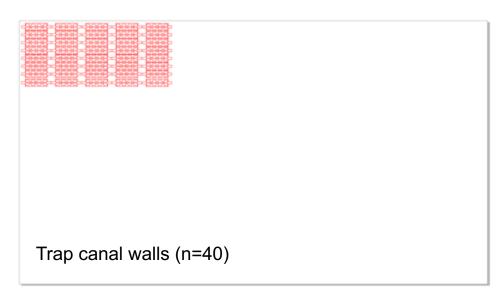




Laser cutting guide > 1/8" acrylic

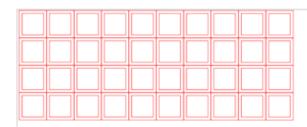
(2 files)



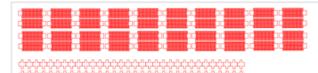


Laser cutting guide > 1/16" acrylic

(2 files)



"Square within a square" for stop pieces and canister bottoms (n=40)



Trap mesh pieces (n=20)

Crosses (n=64)

Quattroport operation checklist

- Set up 4 ports, check for snug fit of 3D printed joints, put parafilm/tape between trap and 3D printed parts, if needed to achieve snug fit. Keep trap doors open with wood dowel.
- Turn on air pump switch

 Flow meter reads "15" for air. CO2 flow meter reads: "0". Check that the tripod is level.
- Attach the 3D printed slider piece of the 4 canisters of mosquitoes (n=20 females per canister) to the 4 starting positions of the assay. Make sure these are snugly attached, place parafilm between start position and 3D slider piece if needed. Insert wood dowel into circular hole on sliding door, THEN remove black screw, record room humidity and temperature. Acclimate mosquitoes for 10 minutes (with air only, No CO₂).
- To start trial, set timer for 5m30s. Start timer, turn on CO₂; the level on the right flow meter should be set to "30" to deliver 1% CO2. Place stimulus in stimulus chamber.
- After 30s of CO₂ flow, remove wood dowels to allow sliding doors to open and release mosquitoes into the assay.
- After 5 minutes, turn off CO₂ off. Flow meter will read "0". Close all 8 sliding doors, 4 in front of the traps, 4 in front of the star canisters, to keep mosquitoes in their position.
- Count mosquitoes in each section of the assay before vacuuming. Record
 the number of dead mosquitoes in the start canister. All mosquitoes that
 have left the start canister are considered "activated", whether or not they
 entered the trap. Mosquitoes that entered the attraction trap are considered
 "attracted".
 - If there are too many mosquitoes in the trap to count easily, label the trap with tape, and add a screw to secure the sliding door in the closed position. Then, carefully remove the trap from the assay (keeping the 3D stop and slider securely fitted on either end of the trap). Don't forget to vacuum the mosquitoes out of the start canister and flying tube before removing the trap. Bring the trap into the cold room, and count the mosquitoes after they have been immobilized.



QUATTRO-PORT

Experiment:
Date:
Hatch date:
Starvation time:

Trial #	Port #	Stimulus	# in trap	# in flying tube		# in start canister		
_	1							
	2							
	3							
	4							
Notes:					Humidity	,		
					Temp:			
					Pressure	:		
Trial #	Port #	Stimulus	# in trap	# in flying	tube	# in start caniste		
	1							
	2							
	3							
	4							
Notes:					Humidity			
					Temp:			
					Pressure:			
Trial #	Port #	Stimulus	# in trap	# in flying tube		# in start canister		
	1							
	2							
	3							
	4							
Notes:				Humidity			_	
				Temp:				
				Pressure				