BAS HoneyComb™

September, 2002

Part MF-9096 INSTRUCTION MANUAL

HoneyComb Fraction Collector

Bioanalytical
Systems, Inc

2701 Kent Avenue
West Lafayette
Indiana 47906

www.bioanalytical.com

MANUFACTURER'S NOTE

This instrument, either wholly or in part, is manufactured for research purposes only. Use for medical diagnosis is not intended, implied or recommended by the manufacturer. Use for this purpose and accountability for the same rests entirely with the user.

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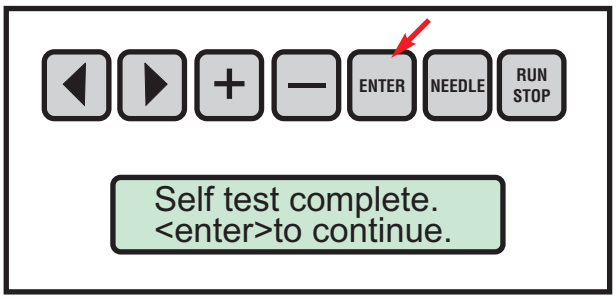
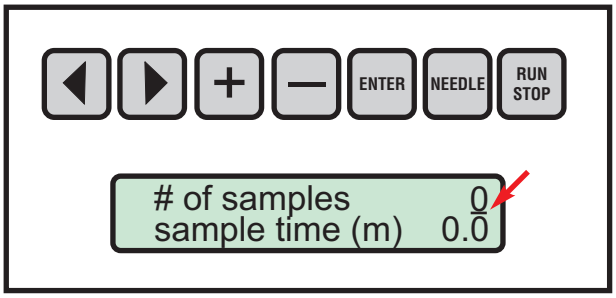
HoneyComb™ Quick Start

September 2002

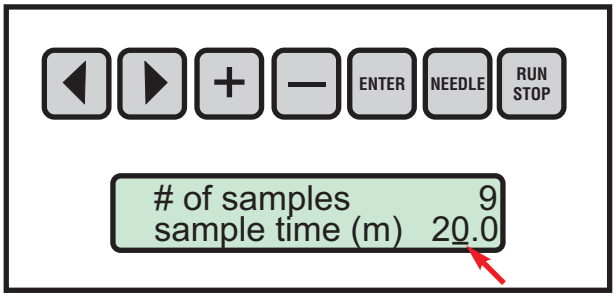


Turn power on (back panel). After the initial powerup of the fraction collector, you will see a message on the screen. This message tells you the collector has finished its internal testing and asks you to press the **ENTER** button (indicated by the arrow) so you can continue with setting up a method on the HoneyComb.

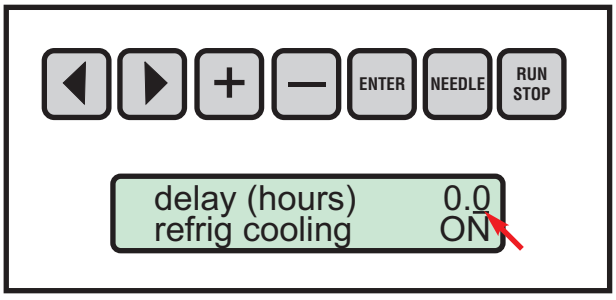


After you have pressed the **ENTER** button (one time), you'll see the next message. This allows you to set the number of samples to be collected, and then to set the time interval between samples. If you have used the HoneyComb before, the last method entered on the instrument will be retained and displayed. Otherwise, all values will be set to zero for initial use. Note the position of the cursor (arrow) which indicates the values you can change. Pressing the **ENTER** key will move to the next cursor position.

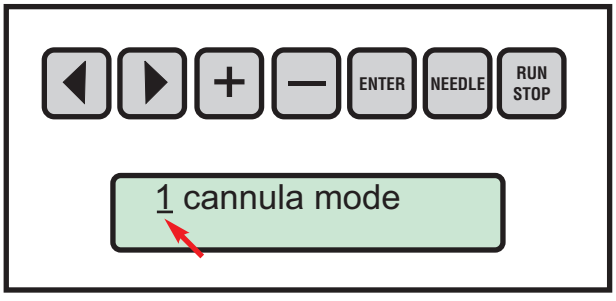
Note! *You will receive a warning message if you enter a value larger than 48 samples. Press ENTER to accept this warning and enter a larger number as needed. Just remember you will have to unload the filled vials and reload with empty sample vials before the carousel begins delivering samples for vials 48 and higher.*



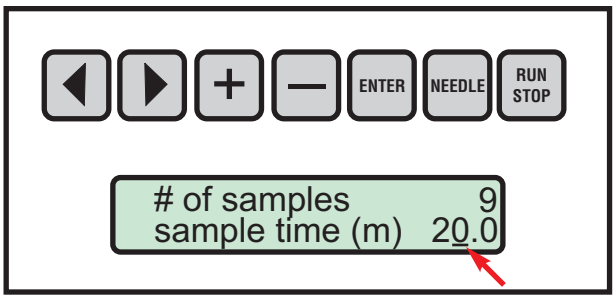
Use the + and — buttons to increase or decrease a value. Use the ◀ and ▶ buttons to move the cursor (see arrow) to a different position. For example, while the cursor is in the position indicating the number of samples, press and hold the + button until the number reads 9 samples. Use the **ENTER** button to move down to the next line. Use the ◀ button to move the cursor left of the decimal point, then hold down the + button until the number reads 20.0. You will now collect 9 samples with an interval of 20 minutes between samples.



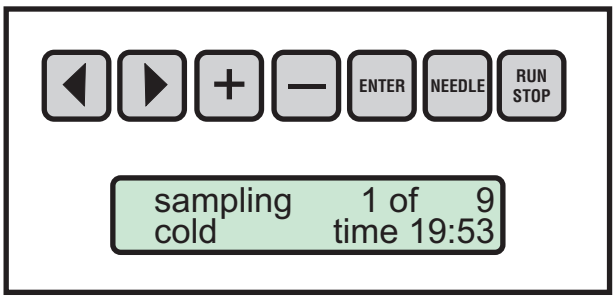
Press the **ENTER** key to move to the next screen. Here you can enter a delay as small as 0.1 hours. The delay will keep the needle in the waste position for a set period of time before it moves to the first vial position and starts counting down the interval you had set in the previous step. Use the same keys as before to set the delay, or just press **ENTER** to go to the next line. The next line will read REFRIG COOLING when it is first powered up and the refrigeration is beginning to chill the vials. After approximately 20 minutes, this message will change to COLD, indicating the collector has reached the preset refrigeration temperature of 3°C.



Press the **ENTER** key to move to the next screen where you can specify you are using either 1 or 2 cannulae (needles) for collection. If you specify 2 cannulae, the collector will advance 2 vials at a time. If you specify 1 cannula, it will advance 1 vial at a time. To help you sort out the vials afterwards, you can refer to the color coding on the collector carousel which uses alternating black or white bars. All of the white bars correspond to one cannula, while all of the black bars correspond to the other cannula.



Press the **ENTER** key again and you will return to the intial screen where you can review or change any of the settings you see. Continue to press ENTER to scroll to other screens you want to change.



To start a collection, press the **RUN/STOP** key. You will see a new screen which indicates which vial (or which pair of vials) is now being collected, and also provides a countdown indicating how long until the collection is completed. When the countdown is finished, the collection cannula will rise and the carousel will advance to the next vial position.

If you had selected a DELAY time, the countdown will begin with this delay time before the cannula advances to the first vial position.

Note! *If the needle carrier is not rotated over the W position on the fraction collector, the unit will not start. Please consult the full manual for instructions on installing the needle.*

Note! *To stop the fraction collector at any time, press the RUN/STOP button. The needle will return to the waste position (W on the collector carousel) and all counters will be reset. If you start again, the collector will return to vial position 1 and start from the beginning.*

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Section 1. Introduction

The BAS HoneyComb™ is a refrigerated fraction collector designed to collect samples from microdialysis or ultrafiltration probes at low flow rates (normally 1-2 µL/min). It may also be used to collect fractions eluting from a microbore chromatography column (aqueous mobile phase) at flow rates less than 200 µL/min. The HoneyComb is part of the BAS Bee family of products for in vivo sampling, although hymenoptera are generally not among the animal subjects studied.

Product Description

The HoneyComb uses a numbered carousel to suspend sample collection vials in a refrigerated "cold canal." The carousel rotates when advancing vials into position under a sampling needle. The needle then descends until it touches the bottom of the vial. Fluid is delivered by direct contact with the vial bottom. The needle uses a non-coring point and air bleed cannula to assist septa penetration and filling. The swept volume of the sampling needle is 7.1 µL. A spring-loaded mechanism on the needle enables contact with the bottom of each vial. The HoneyComb can be used to collect fractions from one source or from two sources simultaneously in adjacent collection vials. The carousel is removable for transport and storage of vials. The needle is fastened to a needle housing which may be rotated by hand to a standby position when removing or installing the carousel. Fraction collection methods may be entered and displayed using the instrument front panel. Control of the vial advance may also be surrended to an external device via rear panel terminal connections.

DANGER! CAUTION! The needle assembly rises and descends as part of its normal operation. **NEVER** place your finger inside the needle assembly while the instrument is turned on.

Features

- One or two sample cannulas
- Sample vials refrigerated to 4 °C
- Open architecture permits vial removal and replacement during collection
- Uses sealed or open vials or combination
- Loaded carousel may be removed and stored
- Simple method setup via front panel keypad and LCD display
- Remote control via Queen Bee Intelligent Syringe Pump or rear panel input
- Optional method delay allows rest of system to equilibrate
- Unit may be slaved to Pollen-8 OnLine Injector for collect/inject operation
- Simple maintenance and replacement of sampling needle
- Sampling needle has small internal volume (7.1 µL)

Components

The HoneyComb is shipped in a single cardboard box. The contents include one of each of the following components:

- HoneyComb instrument
- Standard carousel
- Instruction Manual
- Power cord
- Waste tube
- Needle assembly in a small shipping container

If you have purchased this instrument as part of a BAS Microdialysis System, look for accessories such as sample vials, seals, spare carousel, crimper, etc. in a separate shipping box.

Shipping Damage

If there is damage to the shipping box or its contents upon arrival, please contact BAS immediately and in writing. There is a limit of 30 days after our shipping date to report any shipping damage, loss or omissions. It is important that you advise us, in writing, about these types of problems. Include your name, address, telephone/FAX/E-mail, and the model and serial number of the instruments. You can send this report to:

E-mail:	bas@bioanalytical.com
FAX:	765-497-1102
Phone:	765-463-4527

Normally, BAS shipments are fully insured and an insurance fee is listed on the invoice unless you or your organization have specifically refused to accept insurance on the shipment. If you have ordered BAS shipping insurance, the damaged item will be promptly repaired or replaced at our option. If you have not ordered BAS shipping insurance, you will have to contact your own insurance company to negotiate damage compensation.

Section 2. Installation

Environment

Place the HoneyComb on a hard, level surface with clearance not less than four (4) inches on all sides. Do not place it next to fans, vents or air flow from adjacent devices. Do not place it in direct sunlight. Temperature is maintained at 4 °C unless ambient temperature exceeds 35 °C. The waste tube on the underside of the instrument should be directed to an appropriate receptacle. This instrument uses a fused, self-sensing power supply. There is no need to change fuses or make other power-related adjustments. The instrument can be plugged into a standard wall outlet using the style of cord appropriate to your region.

Sample Vials

The standard HoneyComb carousel is designed to use round-bottomed 6 x 32 mm borosilicate glass vials (BAS part no. MF-5270). **Placement of vials other than 6 x 32 mm glass vials in the standard carousel may result in damage to the needle assembly.** Another carousel is available for taller 6 x 35 mm polyethylene sample vials as a separate accessory. This optional carousel is labeled “plastic vials.”

The HoneyComb will deliver fluid into open vials, sealed vials or a mixture of open and sealed vials in the same carousel. For sealed vials, BAS recommends only our part no. MF-5272, which seals with a soft silicon center that is Teflon-coated on both sides. Use of other seals may result in damage not covered by the warranty.

Vial Capacity

Due to the open architecture of the fraction collector, vials may be removed and replaced during fraction collection, thus extending the total number of fractions which can be collected. Vials should be maintained in all carousel positions to maintain optimum cooling.

For 1 Cannula Mode

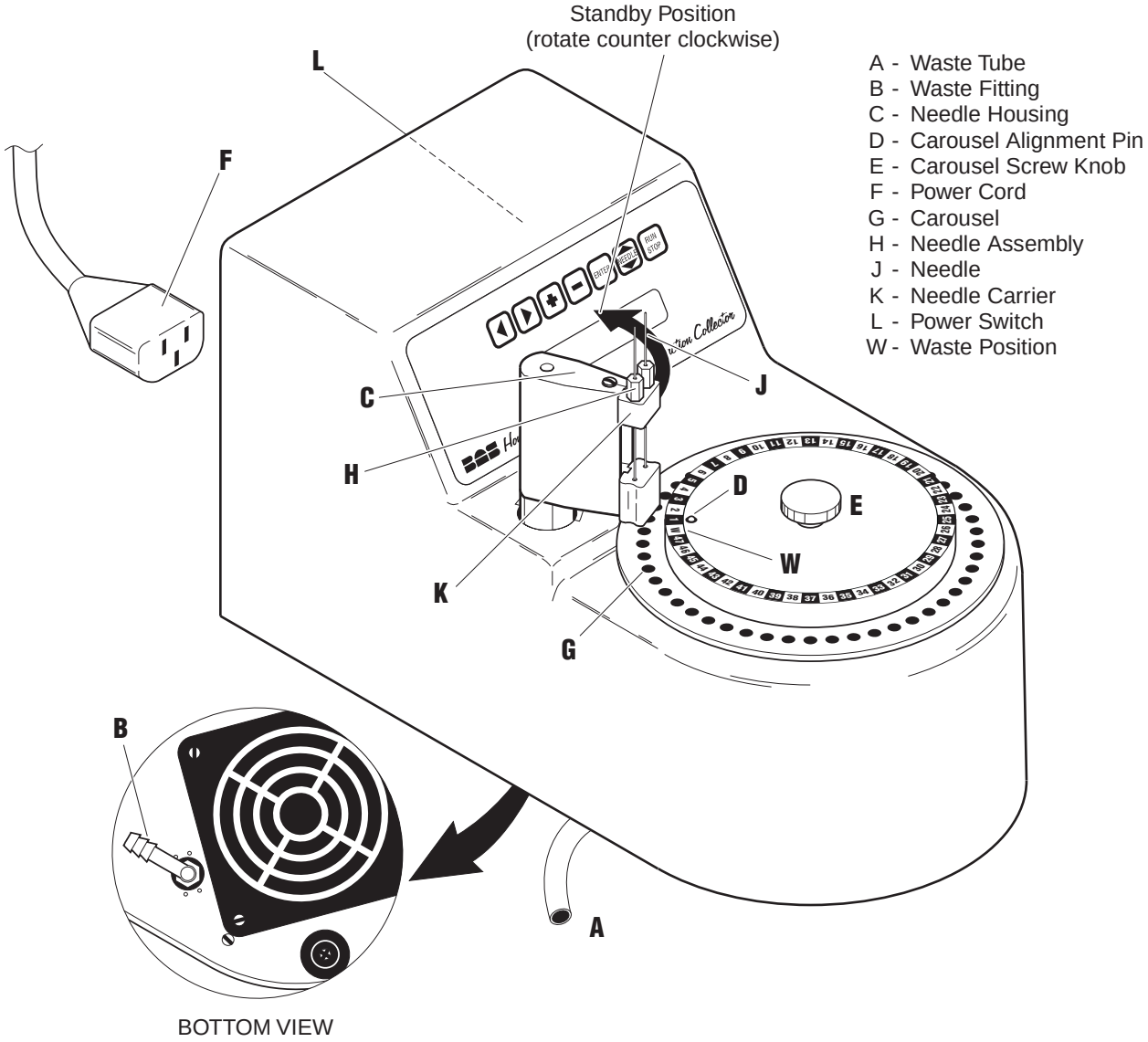
The capacity of the carousel is 47 sample vials. There is also a waste **W** position on the carousel which may be used for another vial. The carousel always advances to the **W** position after power-up or after completion of a collection method. If there is no vial in that position, the needle will drain fluid into the cooling canal and waste tube. If you wish to collect dialysate during a delay period prior to the microdialysis experiment, a vial may be placed in the **W** position. After the collection protocol begins, this waste vial may be replaced with a new vial and used to collect sample number 48. Any interruption in the fraction collection sequence will result in travel of the sampling needle back to position **W**.

For 2 Cannula Mode

The carousel advances two positions for each sample collection. This allows simultaneous collection of two samples in adjacent vials. One sample is always collected in odd-numbered vials (black), while the other sample is collected in even-numbered vials (white). Carousel capacity is 23 pairs of samples. Positions 47 and **W** are waste positions which may be used for an additional pair of vials. The carousel always advances to positions 47 and **W** after power-up or completion of a collection method. If no vials are in

these positions, the needle will drain fluid into the cooling canal and waste tube. If you wish to collect dialysate during a delay period prior to the microdialysis experiment, vials may be placed in positions 47 and **W**. After the collection protocol begins, these waste vials may be replaced with new vials and used to collect sample number 24. Interruption in the fraction collection sequence will cause the sampling needles to return to positions 47 and **W**.

F1. Parts identification



Setup

1. Locate the waste tube **A**.

2. Make sure the power cord is unplugged. Gently turn fraction collector on one side and attach the waste tube to the barbed plastic fitting **B** on the underside.

3. Rotate the waste fitting until the waste tube is directed to the selected disposal site. Fluid coming from this tube will be waste perfusion fluid and condensate.

4. Return the HoneyComb to an upright position.

5. Remove shipping tape and rotate needle housing **C** to the standby position.

6. Place the carousel **G** over the cold canal. Align the hole in the carousel with pin **D**. Press carousel down and tighten the mounting screw knob **E** until carousel is firmly seated.

7. Plug the power cord **F** into the HoneyComb back panel and then into a power outlet.

8. Turn on the power switch **L** located on the back panel next to the power cord. CAUTION: The carousel will rotate during self-test.

9. Wait for the system self-test to be completed (about 15 seconds).

10. Press the ENTER button once.

11. Rotate needle housing **C** over the carousel until the needle housing clicks into place.

12. Press the NEEDLE key. The needle carrier **K** will descend over carousel positions 47 and **W**.

13. Switch the power off.

14. Remove the needle assembly **H** from the shipping container in the accessories bag.

15. Hold the needle housing **C** to prevent rotation. For 1 cannula operation, insert needle assembly **H** into the needle carrier **K** hole located over the **W** position. Screw the needle assembly into place until the hex shoulder bottoms against the top of the needle carrier **K**.

16. For 2 cannula operation, screw a second needle assembly into the needle carrier hole located over position 47.

17. Be sure that the needle assemblies are screwed all the way down. If not, the needle tips may not touch the bottom of the vials.

18. Switch the power on. **CAUTION:** The needle will rise and the carousel will rotate during self test.

19. Rotate the needle housing **C** to the standby position. Fill all the vial positions using appropriate vials (see “Sample Vials” section of this manual). Place plastic vials only in the optional carousel labeled PLASTIC VIALS.

20. Use the keypad to set your fraction collection method.

21. Rotate the needle housing **C** back over the carousel before starting your method. The unit will not operate unless the needle housing has clicked into place over the carousel.

Vial Carousel

Before removing the vial carousel, make sure that the needle is up and the needle housing is rotated over to the standby position. Place one hand on the flat top of the carousel while unscrewing the central knob in a counterclockwise fashion. Once disengaged, the carousel will pop up. It can be removed and transported by holding on to the knob. If the carousel is placed on a hard surface, the vials will rise slightly out of their positions so that they are easier to grasp. To replace the carousel, align the hole **D** with the pin on the HoneyComb. Press the carousel down with one hand while turning the knob clockwise to lock it in place. It is important to make sure that the knob is tightened completely. Otherwise, the vials will ride too high and may interfere with needle movement and positioning.

Section 3. Collecting Samples

Key Functions

◀ and ▶ Move the cursor left and right within a line.

+ and − Increase or decrease a numerical value within a line.

ENTER Advances to the next method line. Use this key to quickly navigate and review the three screens.

NEEDLE Raises or lowers the sampling needle. This will not function unless the needle housing is directly over a vial position in the sample carousel.

RUN STOP starts or stops a method. Once started, the unit begins the delay time and sampling interval selected in the method. When a method is completed or stopped, the sampling needle always returns to the **W** position.

Creating a Method

The HoneyComb will collect microdialysis fractions according to a simple protocol which can be set using the front panel keypad and LCD display. When the power is turned on, it will display the last protocol used prior to being powered off.

There are five parameters involved in setting up a method:

1. Number of Cannulas
2. Number of Samples
3. Sampling Interval
4. Delay Time
5. Refrigeration: On/Off

NUMBER OF CANNULAS. In 1 cannula mode, the carousel is advanced one position after each collection. A single needle is used to collect samples for every vial. The needle should be installed in the mounting hole located over the **W** vial position. In 2 cannula mode, the HoneyComb permits collection of two samples simultaneously in adjacent vials. The carousel advances two positions after every collection. One sample is always collected in ODD-numbered vials, and the other sample is always collected in EVEN-numbered vials. Use the + or − keys to toggle between 1 cannula mode and 2 cannula mode.

NUMBER OF SAMPLES represents the number of samples which will be collected by each cannula during the protocol. Normally this is a number less than the 47 numbered positions on the carousel for 1 cannula mode or 23 for 2 cannula mode collection. Because of the open architecture design of the BAS HoneyComb, it is possible to collect into more than 47 vials (or 23 pairs). Sample vials may be removed after collection and replaced with empty vials. If a number exceeding 47 vials (or 23 for 2 cannula mode) is entered into this field, a warning will be displayed to remind the user that carousel capacity has been

exceeded. The range of samples that may be entered is 1 to 999. Entry of the number 999 will allow collection to continue indefinitely.

SAMPLE TIME represents the time of collection into each vial. It is expressed in minutes and has a range of 0.1 to 99.9. Variable time fraction collection may be set if using the HoneyComb with the Queen Bee intelligent syringe pump, or if timing of the vial advance on the HoneyComb is surrendered to a remote device via the back panel terminal strip.

DELAY TIME represents the time that the needle will remain in the **W** (waste) position prior to advancing to the first vial and starting the sampling interval. It can be used while the rest of the perfusion system is being flushed or equilibrated. It can be used to collect a sample (if a vial is placed at **W**) while the tissue is adapting to the probe. If you don't wish to use a delay time, enter a zero for this value. The range of delay time is 0 to 9.9 hours in 0.1 hour increments.

REFRIGERATION is an option. Use the (+) or (−) keys to turn refrigeration ON or OFF. If you have selected a refrigerated method, allow 30 minutes for the unit to become fully cooled. Refrigeration initiates immediately after selecting ON. If the last method used on the HoneyComb (before power was turned off) was refrigerated, the instrument will begin cooling when power is turned on again. The HoneyComb preset refrigeration temperature is 4 °C. At an average room temperature of 24 °C and 50% relative humidity, it normally achieves this set point in less than 30 minutes. While cooling down, the refrigeration line displays a **"cooling"** message. At 4 °C, this message will change to **"cold."**

Reviewing a Method

Use the ENTER key to quickly scroll through the three screens to review the method. Parameters can be changed any time before starting a method.

Starting a Method

Be sure that the needle housing **C** is rotated over the carousel **G** until it clicks into place. The HoneyComb will not operate unless the needle housing is over the carousel. Press the RUN STOP key to begin the method.

End of Method

When the collection method is complete, the needle will automatically lift out of the last vial, the carousel will rotate to **W** position, and the needle will descend. To remove vials, raise the needle by pressing the NEEDLE key. Rotate the needle housing **C** to standby position.

Interrupting a Method

Press the RUN STOP key at any time to stop a method. The needle will lift out of the vial, the carousel will return to the **W** position, and the needle will descend. THIS ENDS THE METHOD. The method cannot be resumed from the last vial position.

Section 4. Remote Control

Rear Panel Terminal Strip

Communication with the HoneyComb is accomplished via a rear panel terminal strip. There are also D connectors labeled for "BAS Communications" and RS-232C. These D connectors are reserved for future options: do not attempt to control the HoneyComb through them.

The rear panel terminal strip provides 4 inputs, 4 outputs and 4 ground connections. All inputs and outputs are open-collector, active low, tied high to 5 V. All inputs are pulse inputs which are activated by a high-to-low transition. Input signals must stay low for at least 1 second before returning to high state. Input signals must be capable of sinking 1.5 mA. Low is detected below 0.5 V. High is detected above 1.9 V. Outputs are initially inactive (high). Active levels are ground to allow flexibility in connecting to most common types of inter-instrument signal sinks. Outputs can source 1 mA at 5 V and can sink a max. of 35 mA.

RUN	Input pulse. Starts a method which has been programmed from the front panel. Same as pushing the front panel RUN/STOP key when not running.
NEXT IN	Input pulse. Starts sequence in which needle rises, carousel advances to next vial and needle descends. For 2 cannula mode, the carousel advances two vial positions. This input is active only when the HoneyComb is in remote mode (all front panel inputs = 0).
STOP	Input Pulse. Fraction collector stops unconditionally. Needle rises. Carousel returns to **W** position. Needle descends. Same as pushing RUN/STOP button on front panel during method execution.
AUX IN	Input Pulse. Reserved.
RUNNING	Output Level. Indicates that a run is in progress. This signal goes active (low) and remains this way until method is completed.
NEXT OUT	Output Pulse. Indicates start of sequence which advances next vial into position.
AUX OUT 1	Output. Reserved.
AUX OUT 2	Output. Reserved.
GROUND	Reference. A digital ground reference level. There are 4 ground terminals.

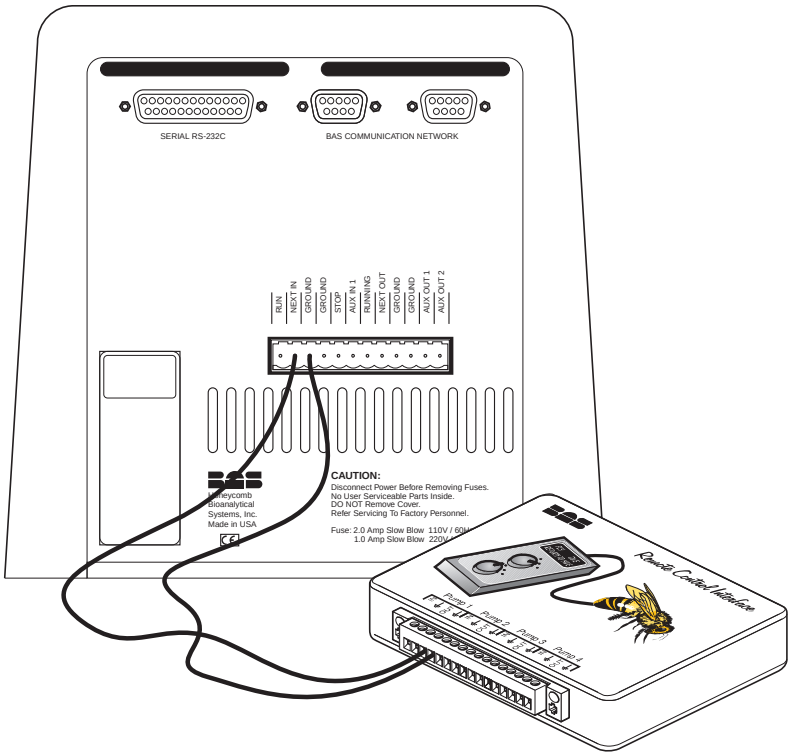
Setup for Remote Operation

To prepare the HoneyComb for remote operation, enter a zero (0) value for DELAY, NUMBER OF SAMPLES, and SAMPLE INTERVAL. Choose the number of sample cannulas desired. Turn refrigeration on or off as desired. Once all inputs have been entered, press ENTER to place the HoneyComb in remote mode. The screen will display REMOTE. To stop the remote mode, press the RUN STOP key. Enter non-zero values for NUMBER OF SAMPLES and SAMPLE INTERVAL to return control to the front panel.

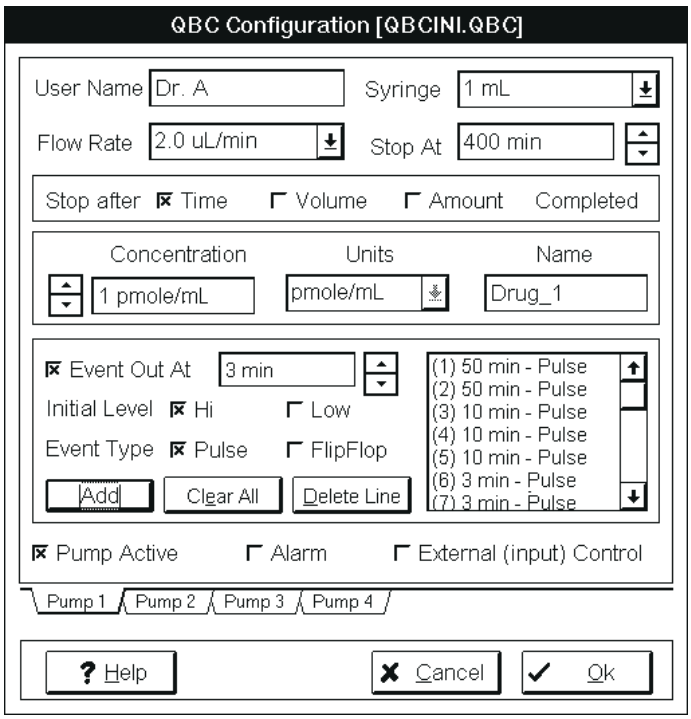
Queen Bee Syringe Pump

To create collection times other than the set interval on the HoneyComb front panel LCD, use the Queen Bee Syringe Pump. It will be necessary to make two connections to the terminal strip, using good quality wire. First, turn off the power to the HoneyComb. Strip the insulation from about 1 cm on both ends of two pieces of wire. The wire should be sufficiently long to reach from the back panel of the HoneyComb to the Remote Control Interface on the Queen Bee. Using a screwdriver, open up two connectors on the strip labeled NEXT IN and GROUND. Insert one piece of wire in each position and tighten the connector with the screwdriver. Examine the Remote Control Interface of the Queen Bee. There are input and output sides to this interface arranged in sets to match each of the four pumps controlled by the Queen Bee. Select connections for the pump (1, 2, 3, or 4) that will be delivering fluid to the HoneyComb. Attach the other end of the NEXT IN wire into the OUT connector on the Remote Control Interface. Attach the other end of the GROUND wire to the GROUND connector on the Remote Control Interface. Use a screwdriver to tighten the screws around the wires on the Interface.

F2. Connecting to the BAS Queen Bee



F3. Queen Bee Configuration Menu



Pull down the Queen Bee Configuration Menu for the pump that will be used with the HoneyComb. Make sure that all other variables describing the perfusion (syringe size, flow rate, limit, and limit type: time, volume, amount) are entered correctly. Any change to these parameters will require setup of a new remote control method on the Queen Bee. Click the EVENT OUT AT box. Then click the INITIAL LEVEL box labeled HI. Then click the EVENT TYPE box labeled PULSE. An X in the box indicates that it is now active. Next, you need to determine the sampling interval that will be used. It will be necessary to enter a value for every vial that will be collected. The value is entered using either the up or down button in the open box next to EVENT OUT AT, or by typing in a value. Note that the units here will be shown as a time, volume, or amount according to the setup of your perfusion method on the same screen. Since it is likely that many of these values will be the same, this task is made easier by clicking on the ADD key to enter the same value again.

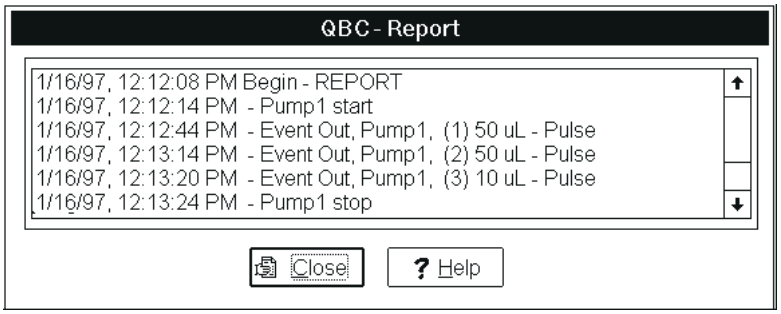
After you have set a value, enter it by clicking the ADD key. The value will appear in the larger box and will be preceded by a number enclosed in brackets, such as (1). The bracketed number represents the sample number. If you keep clicking ADD, the same value will appear while the number in the brackets will increase. To change the value for a particular sample, highlight the line in the method by clicking on it and then use the DELETE LINE key to remove the line. Highlight the line describing the prior sample and then use ADD to insert a new value for the deleted line.

When you are satisfied with the method that you have created, click the OK button at the bottom of the configuration screen and return to the operations screen. Pull down the

configuration menu and select SAVE to retain the file describing this configuration. The file name will have the extension .qbc once saved.

Pull down the configuration screen and open the REPORT window, if desired. Begin the perfusion and the variable sampling interval by pressing the appropriate pump start button at the bottom of the screen (P1, P2, P3 or P4). The progress of the perfusion will be displayed on the bottom line of the operations screen. The history of the protocol, including external events will be displayed on the REPORT screen.

F4. The Report Screen



Example:

A protocol was designed to collect microdialysates from a probe that would be perfused at a rate of 2 µL/min using a 1 mL syringe. Two samples were to be collected at 50 minute intervals to monitor basal levels of a particular metabolite. Then collection would change to 10 minute intervals for a period of 30 minutes. During this period, a drug would be administered to the animal and its effect on the metabolite would be monitored during a 15 minute period in which sampling frequency would be increased to every 3 min. After this period, the metabolite concentration in the dialysate was expected to change very slowly. The collection frequency was decreased to 60 minute intervals for a period of 4 hours.

Method setup would begin by entering the syringe size and flow rate. This protocol could be approached in terms of either time or volume collected. To set up the external output commands on the basis of time, click the STOP AFTER parameter as time. To set up the external output on the basis of volume, click the STOP AFTER parameter to volume. The following chart illustrates how the external input commands would be listed for both approaches:

Sample #	Ext. Output At:	Ext. Output At:
1	50 min.	100 µL
2	50 min.	100 µL
3	10 min.	20 µL
4	10 min.	20 µL
5	10 min.	20 µL
6	3 min.	6 µL
7	3 min.	6 µL

Sample #	Ext. Output At:	Ext. Output At:
8	3 min.	6 µL
9	3 min.	6 µL
10	3 min.	6 µL
11	60 min.	120 µL
12	60 min.	120 µL
13	60 min.	120 µL
14	60 min.	120 µL
Total	385 min.	770 µL

Notes on Remote Control Methods

1. If you enter sampling intervals which represent a volume larger than the volume in the syringe, the Queen Bee will still allow you to proceed. For example, if you have set up a method in which twenty (20) samples of 100 µL each are collected and you have indicated the use of a 1.0 mL syringe, you will have exceeded the syringe capacity by 1,000 µL. Unless you stop the pump and replace the syringe (not recommended for microdialysis!), you will run out of perfusion fluid before your protocol is halfway completed.

2. All values for methods parameters on the HoneyComb must be set to zero (0) before the Queen Bee can take over collection.

3. The Queen Bee controls the syringe pump as well as the variable sampling interval. Once the method is executed, the pump will begin the perfusion and the sampling needle will advance to vial position one (1). There is no preset delay time when using the Queen Bee. The HoneyComb will automatically advance to the first vial.

4. If you have set up a method involving variable sampling intervals controlled by the Queen Bee, sample collection in the last vial will continue until you physically interrupt the collection by pressing the RUN/STOP button on the HoneyComb, or unless you have created a limit for the perfusion within the Queen Bee protocol.

5. You can monitor the progress of the external event output by opening the REPORT window which will record all external outputs as they occur. At the end of the protocol, you can save this report as a disk file which can be later printed.

Control by Pollen-8 OnLine Injector

The HoneyComb can also be used in conjunction with the Pollen-8 OnLine injector when the 10-port valve is configured to alternate between injection into an LC system and collection in a vial. The rear panel terminal strip will also be used for this operation. Please consult the Pollen-8 manual for details.

Section 5. Maintenance

The HoneyComb requires minimal maintenance by the average user. The primary focus for the user should be to maintain the cleanliness of the sampling needle by thorough flushing with filtered, deionized water after each use. Physiological fluids used in dialysis are salty. If these solutions are allowed to dry within the sampling needle, salt crystals will form. Since all fluid paths within a microdialysis system must be flushed thoroughly with water after use, make sure that the HoneyComb is included. Periodic cleaning of the cold canal with a cotton swab is also recommend to dislodge dust. Rinse the cold canal using distilled water in a squeeze bottle to rinse salts into the waste tube.

Replacement Parts

Part Numbers	
MD-1201	HoneyComb Fraction Collector
MW-2304	Spare Carousel for Glass Sample Vials (6 x 32 mm)
MW-2305	Optional Carousel for Plastic Sample Vials (6 x 35 mm)
MF-5270	300 µL Glass Vials, 1000/pkg.
MF-5272	Caps and Seals for MF-5270, 1000/pkg.
MF-5274	Crimper for MF-5270 and MF-5272
MF-5271	250 µL Plastic Sample Vials (6 x 35 mm) 1000/pkg.
MW-2310	Replacement Complete Needle Assembly

Needle Replacement

If the needle bends or breaks, replace the needle assembly (MW-2310) using the following procedure:

1. Stop the instrument. Press the NEEDLE key to raise the needle and rotate the needle housing **C** to the standby position away from the carousel.

2. Switch the power off.

3. While holding the needle housing, unscrew the needle assembly **H** from the needle carrier **K**. Use a 1/4 inch wrench if necessary.

4. Before inserting the replacement needle, switch the power on and rotate the needle housing over the carousel. After the self test is complete, press the ENTER key, then press the NEEDLE key to lower the needle mechanism.

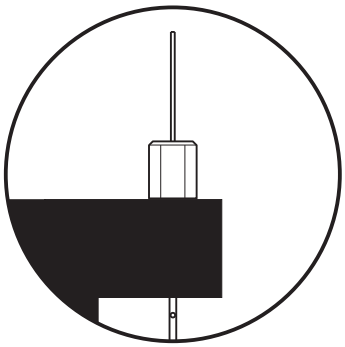
5. Switch the power off.

6. Hold the needle housing **C** so that it does not rotate and insert the needle assembly into the needle carrier. For 1 cannula operation, the needle assembly should be inserted into the hole located over the W position.

7. While holding the needle housing, screw the needle assembly into the needle carrier until the hex shoulder bottoms against the top of the needle carrier **K**. Use a 1/4 inch wrench to gently tighten the needle assembly against the needle carrier. See **F5**.

8. Switch the power on.

F5. Replacing the needle assembly



Troubleshooting Guide

At some time, you may see an error message on the LCD display of the HoneyComb. This message will usually include a few words and an error number. It may also be combined with a message that says “Call BAS.”

If you see such a message, please take a moment to write down the entire message. Before you call, there are some remedies which you may attempt on your own:

1. Turn off the power on the back panel of the instrument.

2. Wait for 30 seconds and then turn the power back on again.

3. If the carousel stopped in the middle of a run before the error message, then the needle should rise and the carousel should advance to **W** before the needle descends again.

This simple remedy will solve the majority of error messages that may occur. The HoneyComb is sensitive to movement of the carousel. If the carousel is moved so that the vials are no longer aligned with the needle assembly, a needle fail-safe will engage to prevent needle descent and error messages will be inevitable. This scenario is resolved by turning off the power. When power is turned on again, the HoneyComb will self-test and re-align the carousel properly.

Symptom	Possible Cause	Remedy
Does not power up.	Power cord unplugged or faulty.	Plug in or replace cord.
	Blown fuse(s).	Replace fuse(s) in power entry module. 2 amp slo blow (110V / 60 Hz) 1 amp slo blow (220V / 50 Hz)
SYSTEM ERROR message.	Diagnostic error.	Power down then power up. If error repeats call BAS.
CAROUSEL ERROR message.	Carousel prevented from moving or is lost.	Remove obstruction. Power down then power up. If error repeats call BAS.
STATION ERROR message.	Needle prevented from moving up or down.	Remove obstruction. Power down then power up. If error repeats call BAS.
	Needle housing rotated away from carousel during a run.	Power down then power up. Rotate needle housing over carousel until it clicks against the stop.
COOLING ERROR message.	Faulty sensor.	Note error and call BAS.
Carousel does not stop with needle over **W** position.	Carousel not aligned.	Alignment pin should protrude thru the alignment hole in the carousel.
	Carousel not tightened down against base disk.	Tighten carousel thumbscrew until carousel is fully down.
Press NEEDLE - nothing happens.	Needle housing not detected over carousel.	Rotate needle housing over carousel until it clicks against the stop.
	Following Power-up ONLY.	ENTER key must be pressed before proceeding.
	# of vials = 0	Number of vials must be > 0 unless controlled remotely.
	sample time = 0	Sample collection time must be > 0 unless HoneyComb is controlled remotely.
Vials do not fit in carousel.	Incorrect vials.	Use MF-5270 - glass, 300 µL, 6 x 32 mm, round-bottom vial
Carousel does not seat against the base disk.	Alignment pin on the base disk does not protrude thru the alignment hole on the carousel.	Line up the hole on the carousel with the pin on the base disk. Push the carousel down so the pin goes thru the hole. Tighten the carousel thumbscrew until carousel is fully seated against the base disk.
	Threaded hole in the shaft is partially filled with foreign matter which prevents the thumbscrew screwing down further.	Remove foreign matter from bottom of threaded hole in the shaft.
	Locking nut on the underside of the carousel is loose.	Screw the nut against the underside of the carousel and bond in place with adhesive.
Needle misses vial septa.	Carousel not aligned.	Alignment pin should protrude thru the alignment hole in the carousel.

Symptom	Possible Cause	Remedy
Needle damaged when contacting bottom of vial.	Carousel not tightened down against base disk.	Tighten carousel thumbscrew until carousel is fully down. Replace Needle Assembly (MW-2310).
	Needle Assembly depth is too low.	Unscrew Needle Assembly a few turns. Replace Needle Assembly (MW-2310).
	Incorrect vials.	Use MF-5270 glass 300 µL 6 x 32 mm round bottom vial with standard carousel. Use MF-5271 plastic vials with optional carousel, MW-2305. Replace Needle Assembly (MW-2310).
Vial bottoms broken by needle.	Carousel not tightened down against disk.	Tighten carousel thumbscrew until carousel is fully down. Check for needle damage.
	Needle Assembly depth is too low.	Unscrew Needle Assembly a few turns.,
	Incorrect vials.	Use MF-5270 glass 300 µL 6 x 32 mm round bottom vial with standard carousel. Use MF-5271 plastic vials with optional carousel, MW-2305. Check for needle damage.
Inconsistent collection volumes in vials. (Needle tip not touching bottom of vials.)	Needle Assembly depth adjusted too high.	Screw Needle Assembly down until the hex shoulder contacts the top of the Needle Carrier.
	Needle stuck - no spring compliance.	Dissassemble and clean Needle Assembly or replace.
	Damaged needle	Replace Needle Assembly (MW-2310).
	Incorrect vials.	Use MF-5270 glass 300 µL 6 x 32 mm round bottom vial with standard carousel. Use MF-5271 plastic vials with optional carousel, MW-2305.
Inconsistent collection volumes in vials. (Back pressure increases as sealed vial fills with fluid.)	Air Bleed Cannula plugged.	Dissassemble and clean Needle Assembly or replace.
Inconsistent collection volumes in vials.	Evaporation	Seal vials with BAS part # MF-5272
	Measurement by weight	Calibrate and test your analytical balance using a standard weight set.
	Perfusion system	Change to a small (500 µL) syringe. This will deliver a more accurate flow rate.
Extra collection volume in vial #48 (**W** position).	Needle returns to **W** position at completion of a run.	Remove vial #48 after collection but before completion of the run.
	Power interrupt	If power is interrupted, needle returns to the position #48.

Symptom	Possible Cause	Remedy
No fluid in vials.	Pump not running.	Check pump.
	Syringe empty.	Fill syringe.
	Leakage.	Check syringe and fluid lines in system.
	Needle plugged.	Remove Needle Assembly and clean needle.
When input pulse sent to rear panel terminal labeled RUN. Nothing happens.	Following Power-up ONLY.	ENTER key must pressed in order to proceed.
	Input signal is High	Input signal must switch to Low (< 0.5 V) for at least 1 second to activate RUN command.
When input pulse sent to rear panel terminal labeled NEXT IN. Nothing happens.	HoneyComb not in Remote mode.	Set all front panel values to zero (0), then press ENTER.
.	Needle housing not detected over carousel.	Rotate needle housing over the carousel until it clicks against the stop.
	Ground wire not connected.	Connect ground wire from control instrument to any GROUND terminal on the HoneyComb.
	Input wire connected to NEXT OUT terminal.	Input wire from control instrument should be connected to NEXT IN terminal on the HoneyComb.
	Input signal is High	Input signal must switch to Low (< 0.5 V) for at least 1 second to activate NEXT vial command.
RUN STOP key does not end Remote mode.	Needle housing not detected over carousel.	Rotate needle housing over the carousel until it clicks against the stop.
Vials rise up in carousel while it rotates	Obstruction	Follow instructions for removing the carousel. Inspect the cold canal and remove any obstructions found.
Needle won't puncture seals	Wrong vial seals	Use only BAS seals (MF-5272).
	Damaged needle point	Replace needle assembly (MW-2310).
Needle does not descend completely or rises again too quickly.	Obstruction in needle carrier	Inspect needle carrier. **Warning:** Do not put your fingers or any other object inside the needle carrier until power is turned off.
	Wrong seals	Use only BAS seals (MF-5272).
	Method	Check method on SAMPLE TIME line. The sampling interval may be set as short as 0.1 minutes (6 seconds).

For other problems contact BAS Service:

Telephone: 765-463-4527

FAX: 765-497-1102
E-mail: bas@bioanalytical.com

Limited Warranty

Bioanalytical Systems Inc. (BAS) warrants that equipment manufactured by the company will be free from defects in material and workmanship for a period of one (1) year from the date of shipment, except as provided hereinafter. Needles, seals, cannulae and other components normally exposed to normal wear are exempt from this warranty. Damage caused by use of the incorrect seals, vials or carousel is exempt from warranty. Claims for shipping damage are invalid unless the company is notified within 30 days of the shipping date. BAS is liable only to the extent of replacement of any items missing, or broken during shipment. BAS will not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever arising from the use of this device. The foregoing warranty is in lieu of all other warranties expressed or implied including but not limited to the implied warranties of merchantability and fitness for a particular purpose.