HARVESTER 96®
Automated Models
OPERATORS MANUAL

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WARRANTY

Tomtec guarantees the HARVESTER 96® against defects in materials and workmanship. Defective materials will be replaced at no charge for a period of one year following shipment. Labor required for warranty related repair will be done at no charge for one year, providing the equipment is returned to our factory for repair. The cost of transportation both ways must be paid by you. Our warranty is valid providing the equipment is utilized within the guidelines of this operator's warranty. Specifically, it is the responsibility of the user to adequately wash and clean the various flow passages within the HARVESTER 96® after use. Damage resulting from failure to thoroughly clean these passages is not covered by this warranty.

This warranty is exclusive and is in lieu of all other warranties, whether written, oral or implied, including the warranty of merchantability and of fitness for any particular purpose. Tomtec's liability is, in all cases, limited to the replacement price of its product. Tomtec shall not be liable for any other damages, whether consequential, indirect, or incidental, arising from the sale or use of its products.

Specifications and data in this publication are believed to be accurate and reliable. However, it is the responsibility of the product user to determine the suitability of the product for a specific application. While defective products will be replaced without charge if promptly returned, no liability is assumed beyond such replacement. Authorization for return must be received from Tomtec Inc. before returning any equipment for inspection or warranty repair.

A completed "Decontamination Form" must accompany all returns.

CAUTION

The HARVESTER 96® is designed for use with various liquids used in cell harvesting. These are primarily aqueous solutions with a near neutral pH. As the pH of the solution moves away from neutral, then various parts may be affected. This depends on the length of time they are in contact. For further information, see the Table of Contents for the section entitled "Solvent Compatibility" of this manual. The user has full responsibility for thoroughly rinsing the system after use. Damage resulting from improper use of liquids and lack of cleaning are the user's responsibility and are not covered by Tomtec's warranty.

Specifications subject to change without notice.

IMPORTANT INFORMATION

Please read the following information regarding your warranty.

The HARVESTER 96®, necessity, has many relatively small flow passages in the wash and overflow manifolds. Salt solutions, if allowed to dry, can cake and clog these passageways. In some cases, the manifolds would require complete disassembly to clean them. This is a costly repair which is not covered by your warranty.

This problem may easily be eliminated by cleaning the Harvester after each use. Damage due to failure to properly clean the Harvester is not covered by your warranty.

General Operational Note

The Harvester 96 is basically designed to use a vacuum source (house vacuum or separate vacuum pump) to pull liquid (water) through a glass fiber filter mat, either as a separate filter mat or filter plates. This liquid may be radioactively "hot" or "cold". Vacuum pumps are designed to only handle air. They cannot handle liquid. Tomtec supplies our Autotraps. These are vacuum traps that separate the liquid water from the air steam that is carrying the liquid. Again this air stream and associated liquid, may be radioactively "hot". Depending on the level of radioactivity, it may require separate disposal. Thus 2 Autotraps may be desired to separate the waste.

Each Autotrap has a magnetic switch that is sealed against liquid damage, to tell the operator when the Autotrap is full, and must be emptied. The question is how is this switch to be used? It's sole purpose is to stop everything when the switch is activated by the liquid level within the Autotrap.

While this is the simple solution, it may not be from an operational viewpoint. Tomtec, nor others, have done any testing whereby the Harvesting process was interrupted and then restarted. It is questionable whether any such testing would be conclusive for all applications.

Tomtec has chosen the following method of operation. Each Autotrap holds about 11 liters of liquid. The high level float is set to actuate at a liquid level of 6 liters. It does not stop the program that is in operation. It does prevent the operator from starting another program without emptying the Autotrap. The remaining 5 liter capacity in the Autotrap should accommodate the balance in most programs. Notice the word "should". This implies a risk the operator must be aware of. The Harvester 96 is supplied with two 9 liter wash buffer bottles. On some Harvesters we supply 2 Autotraps i.e. "Hot" and "Cold".

How do you protect the vacuum pump against water damage? Particularly if it is "Hot" waste. The most economical method is common sense from the operating personnel. By being aware of the situation, the operator knows the relative volumes being used.

There is concern about the end results, if you interrupt the harvesting process and then restart it. However, if positive overflow protection is desired, Tomtec can simply supply a small safety trap or an overflow trap if required. This is located at the input to the vacuum pump. If the float switch in the safety trap actuates, it shuts down the harvesting process regardless of completion, before damage can occur.

HARVESTER 96®

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REQUIREMENTS

To install the HARVESTER 96®, check that the following items have been delivered or are available.

1 – Harvester 96®

2 – Wash reagent bottles

Quick connect tubing (wash reagent bottles to Harvester)

1 – Air Line Assembly

2 – Air Line Assembly

1 – Power Cord

1 – 1/2" ball value assembly

OPTIONAL EQUIPMENT

1 – Vacuum trap, Tomtec's Autotrap recommended

1 – Vacuum line filter (delivered with the Autotrap)

1 – Drain tubing (delivered with the Autotrap)

1 – Vacuum tubing (1/2" ID, steel reinforced, delivered with the Autotrap)

Quick connect tubing (wash reagent bottles to Harvester)

INSTALLATION

Vacuum: 20+ inches of Hg preferred (The unit can operate at lower vacuum.)

Air: Greater than 5 psi, normal lab air supply Power: 115/240 Vac 50/60 Hz

All connections in Fig. 1 are shown using the optional Autotrap(s) from Tomtec.

1. Position the vacuum trap as close as possible to the Harvester on the lab bench.
   a. Connect the Autotrap canister(s) to the Aspirate Out on the Harvester.
   b. Connect the Autotrap control module(s) to the vacuum source.
   c. Connect regulated air to the top of the control module(s). ¼" clear plastic tubing with snap connectors.
   d. Plug in the four pin connector(s) cable from the Autotrap control module(s) to the back of the Harvester.

For further tips, see section: Vacuum Considerations

2. Position the wash reagent bottles close to the Harvester on the lab bench.
   a. Connect the quick connect tubing between the wash reagent bottles and the Harvester, first at the Harvester, then at the bottle.

b. Connect regulated air from the back of the Harvester to the lids of the wash reagent bottles.

3. Connect unregulated air at more than 5 psi to the back of the Harvester (Air In).
4. Connect the power cord, and turn on the Harvester.
5. Set the air pressure. With power on, press the" prime" button. Scroll until the display reads:

"Display Pressure"

Press the RUN button. Adjust the regulator knob on the back panel until the desired air pressure is set. Press the STOP button.

6. Press the F1 button. The display will read "Prime Wash Buffer". Press RUN. Then use the value button to select either L (Left) or R (Right) wash reagent bottle. Press "RUN" to enter selection. Place the prime pan on the stage, and raise the stage. Press RUN and hold it until wash reagent is coming out of all orifices. Press STOP to return to standby mode.

7. Check that any overhead shelves do not restrict space needed to open the head on the Harvester.

8. The Harvester is now ready to operate. This assumes the vacuum traps have been connected and the vacuum is on. Place a filter mat in position on the upper head assembly. Well A-1 will be in the front left hand corner of the MII and the rear right hand corner on the MIII, of both the microplate (below) and the filter mat (above). Cells will be harvested to the bottom side of the filter mat.

   Note: That a long edge guide is provided to position the filter mat. This allows precise positioning each time. Close the head, and pull the handle down into its locked position.

OPERATIONS

The Harvester is designed to provide the utmost flexibility in cell harvesting. Parameters are individually controlled to enable the operator to set the optimum conditions for any protocol. An understanding of the principles of operation will allow operators to use their own creativity in achieving the desired results.

The Harvester is designed to harvest from receptacles in the 8 X 12 array on 9mm center-to-center distance of standard microplates. These may be individual microplates or microtubes arranged in the microplate format. When using microtubes, it is necessary to extend the aspirate needles with adapter lengths of tubing. These are available as optional accessories from Tomtec.

There are five solenoid valves controlling the HARVESTER 96® functions:

Wash Valves: Control the amount of wash from the left or right reagent containers to the wash manifold.

Vacuum Trap Valves: Open the vacuum aspirate line to direct the wash to either the "hot" or "cold" trap.

Overflow Valve: Connects the overflow ring around each well to the "hot" trap.

Using two traps allows the user to separate the waste stream to either a "hot" or "cold" receptacle, thereby reducing the amount of radioactive waste to be disposed of.

The microprocessor based control system allows 16 different programs to be created and saved using combinations of these valves.

CHANGING THE VALVE TO THE HOUSE VACUUM

Conventional lab vacuum valves are of two types: a quarter turn shut-off cock and a bonnet type valve (several turns). Both are designed with small flow passageways to restrict loss of vacuum in the system if a valve is left open unintentionally. These restrictions will inhibit the operation of the Harvester. Tomtec supplies a ½" ball cock shut-off valve. It is supplied with 3/8 NPT fittings to mate with your laboratory fitting.

The valves can be changed with minimum impact on the house vacuum. If your valve is the ¼ turn shut-off cock, simply plug the opening. Turn the handle to the "on" position. This will allow it to clear the bench top as you unscrew it. Quickly exchange it with the valve we have supplied. Again, it may be necessary to turn the handle to the "on" position so it may be rotated as it is screwed in place. Plug the open end with your thumb or a cork until it is screwed in place and can be turned off.

If your lab has a bonnet type valve, it may be necessary to unscrew the bonnet (top) to allow the valve body to be unscrewed. Again, plug the opening with a cork or a piece of cardboard to minimize the impact on the house vacuum while changing the valve.

AIR PRESSURE CONNECTION

Tomtec supplies an air line to go between the lab air supply shut-off cock and the Harvester. Be sure to tighten the clamps since that connection will withstand full air line pressure. If you do not have laboratory air, there are several alternatives. Please call Tomtec for recommendations.

WASH REAGENT CONTAINERS

The wash container is supplied with a quick connect liquid line. When disconnecting, always do so at the bottle. That fitting has the shut off valve. Do not disconnect at the Harvester. There is not shut off there.

Connect the air line from the back of the Harvester (Air Out) to the quick connect on the cap of the wash container. This will supply the regulated air pressure (0 to 5 psi) to the wash container. The slides of the wash container should slightly bulge when pressurized. If they do not, check to see that the cap is tight and the air line is fully connected (snaps in place).

FILTER HEAD ASSEMBLY

The filter head used a single handle to clamp and lock the filter mat in position. Two edge guides are used to accurately position the filter mat. Double "O" rings seal each aspirate line to the filter mat.

An easy check on the operation of the Harvester is to place a dry filter mat in position and close the head. Then run one or more wash/aspirate cycles. Open the head, and examine the filter mat. There should be 96 wet circles surrounded by dry corner

quadrants. You will see the impressions of the “O” rings, top and bottom. This is a quick method of checking that all “O” rings are sealing properly and that all aspirate lines are open and not clogged.

There are high localized pressures at the clamping points of the head handle. After repeated use, some wear may occur at these points. This may reduce the clamping pressure and resultant “O” ring sealing. The clamping pressure is controlled by adjustment of the cam rollers on the right and left side of the head. Each side should be adjusted uniformly to maintain an even head pressure.
See Technical Note: Adjusting Head Clamping Pressure.

MICROPLATE NEST AND CARRIAGE ASSEMBLY

Individual microplates are held in an adjustable nest located on the microplate carriage assembly. The four edge guides should be positioned to hold a microplate or rack of microtubes in the correct position on the carriage. Remember that the wash jets should be near the top of a well. The microplate or microtubes must be positioned high enough so that these tiny jets are within the wells. If the aspirate tubes do not touch the bottom of the wells, they may not aspirate all of the cells from the well. Be aware that the tip assembly will displace some volume as it enters the well. This is important if the microplate well is full (i.e. near 300 microliters) at the start. If the well volume on the sample plate is less than 200-250 microliters, then any displaced volume should not cause the well to overflow. Be aware of possibility, and check to be sure.

The push buttons on the operator’s panel are divided into three sections – Parameters, Program, and Status. A single line display is used to communicate with the operator. The buttons have the following functions:

Parameter

Cursor: Scrolls through the program steps (going right). Change program name (going left).

Value: Changes the value of any parameter accessed by the cursor.

Program

Scroll: Scrolls through the available programs of prime functions.

Select: Press it to put the HARVESTER 96® in the run mode.

Set Up: Press it to put the HARVESTER 96® in the programming mode.

Status

F1: Accesses three functions:

- Prime Wash Buffer
- Display Pressure
- Prime Aspirate Line

F2: Non functioning button.

Run: Starts a selected sequence or program.

Stop: Stops any sequence or program. Returns to standby mode from setup mode.

NOTE: If stopping a program in the midst of its execution, the display will read "Paused". To continue, hit RUN. To abort, hit STOP. The display will read "No <<Cancel Run>>Yes". To abort, hit right cursor. To continue, hit left cursor, which will return you to "Paused" where you can now hit RUN.

PROGRAMMING

The flexibility of the Harvester programs allows the user to create their own harvesting sequence. Up to 16 different programs can be created and stored in memory. Each program may have up to five cycles.

To enter the programming mode, press the SET UP button. Then press PROGRAM SCROLL to advance to the program you wish to change/create. Press the PARAMETER SCROLL button to advance through the parameters and the VALUE SCROLL to set desired variables.

	PARAMETER DISPLAYED	VARIABLE TO BE SELECTED
	Dry Time	Time in Seconds
	Overflow Active	Yes – No
	Cycle Three Repeat	1 to 99
	Cycle 1	
1:	Wash Buffer	R or L
1:	Wash Time	Time in 0.1 Second Intervals
1:	Overflow Trap	Hot – Cold
1:	Soak	Time in 1 Second Intervals
1:	1st Aspirate Time	Time in 0.1 Second Intervals
1:	1st Aspirate Trap	Hot – Cold
1:	Wash/Aspirate Time	Time in 0.1 Second Intervals
1:	Wash/Aspirate Trap	Hot – Cold
1:	2nd Aspirate Time	Time in 0.1 Second Intervals
1:	2nd Aspirate Trap	Hot – Cold
1:	Pause Here	Yes – No

Cycle 2, 3, 4, and 5: The same parameters as outlined for Cycle 1 above may be set with their variable values.

CHANGING THE NAME OF A PROGRAM

The name of a program can be changed to any 12 letter name or mnemonic. Use the PROGRAM SCROLL button to locate the desired program, then the PARAMETER SCROLL button to scroll left. A flashing cursor appears and moves left to each character in the display. Stopping the cursor on a character allows that character to be changed by using the **VALUE SCROLL** push buttons.

DESCRIPTION OF PARAMETERS

Dry Time. At the end of the last cycle in the program, a beep will sound. This indicates the start of the dry time. The vacuum will stay on for the time set in seconds. Opening the head will draw air through the filter mat to assist in drying it.

Overflow Active

Opens the overflow valves during the wash period only. During the time the wash solution is flowing, vacuum is connected to the ring around the top of the well. Should the well overflow for any reason, the excess will be captured by the overflow ring and carried directly to the hot trap. The overflow valves will be opened only during the wash portion of the cycle. It selected, they stay on for one second following the wash to clear the overflow manifold. If both the overflow orifices and aspirate orifices were open simultaneously, it would overload the vacuum source and decrease the harvesting.

Cycle Three Repeat

Sets the number of times Cycle 3 is to be repeated. Will improve the washing function by filling and emptying the wells in rapid succession. Often used with “sticky” cells.

Wash Buffer

Allows the selection of which buffer container is going to be used for this cycle (right or left).

Wash Time

Determines the length of the time the wash valves are open and deliver wash to the wells of the microplate or microtubes. The operator must be careful not to overfill the wells. At 3 psi of pressure on the wash reagent bottles, a wash time of 0.4 seconds

will add about 200-250 microliters to each well. Trial and error will find the correct setting for the desired application.

Overflow Trap

The overflow is always connected to the hot trap. If only one trap is used, it must be connected to the hot trap connection.

Soak

Allows the wash liquid to remain in the wells for the soak time selected before being aspirated. Soak time is adjustable from 0 to 254 seconds in one second intervals. If set to zero, the soak function is bypassed.

1st Aspirate Time

Opens only the aspirate valve for the time period selected. If the 1st aspirate function is not desired in a sequence, setting the time to zero bypasses it.

1st Aspirate Trap

Selects the “hot” or “cold” trap to be used to catch the effluent from this aspirate.

Wash/Aspirate Time

Opens the wash and aspirate valves simultaneously. The wash will enter each well at the top from the four tiny jets. It will wash down the sidewalls and be aspirated up through the filter mat to the trap.

Use care in setting the flow rates during the wash/aspirate. The wash rate into the wells remains constant, determined by the head pressure being used, whereas the aspirate rate is influenced by a change in the available vacuum and loading of the filter mat. If particulate matter is being aspirated, this flow rate will decrease over time. **The flow rate into the wells must be less than the aspirate rate or flooding will occur. If flooding occurs, decrease the head pressure on the wash container.**

If the wash/aspirate function is not desired in this sequence, setting the time to zero bypasses it.

2nd Aspirate Time

Sets the time that only the aspirate valves are open. If a wash/aspirate is used, it should always be followed with a second aspirate of one second (or more) to remove the remaining wash in the lines. Setting the time to zero bypasses this function.

2nd Aspirate Trap

Select the trap is to be used to collect the effluent from this aspirate.

Pause Here

If selected, the cycle stops at this point. It waits until the operator presses RUN to continue on the rest of the program. This function allows manual intervention into an otherwise automatic cycle. There are a number of uses for this function. For instance, it may be desirable to pre-wet the filter mat with an empty plate prior to running the sample plate. An empty tray may be placed on the stage and wash/aspirate in Cycle 1. A pause would allow the operator to remove the empty tray and replace it with the sample tray. Pressing RUN would then execute the rest of the program.

Cycle 2, 3, 4, 5

There are up to five cycles available in each program. Each cycle allows the setting of different values for each of the parameters listed above. If any parameter is not desired, it may be set at zero, effectively bypassing it.

Note: When selecting a program to be run, the right hand character in the display will show the number of cycles to be run. Using the **VALUE SCROLL** push buttons, this value may be changed.

Changing Wash Reagent Bottles

If the program requires a change of buffer, please note that the Harvester requires about 300ml to completely flush the internal manifold. Use the cycle three repeat function to perform the regular wash, then use the two last cycles, with a different wash buffer. This will be as close to a uniform wash as possible. If in doubt, call Tomtec for advice.

SAMPLE PROGRAM

Assume the following sequence of events is desired in a protocol that “Betty” uses for her work. The filter mat is first pre-wet with buffer. The sample is to be aspirated to the filter mat and then washed with buffer. The first part of the aspirate will contain a higher level of radioactivity and must go to the hot trap. On subsequent washes, the radioactivity will be low and may drain into the sink. The final step will be to aspirate a small volume of alcohol through the filter mat to assist in drying.

Since this program will have one primary user, we will change the display from the default setting of “Program 3” to show the user’s name (in this example, “Betty”). We will set the following the variables for the parameters. The wash buffer to be used will be in container A.

PARAMETER DISPLAYED	VARIABLE TO BE SELECTED
Dry Time	10 Seconds
Overflow Active	Yes
Cycle Three Repeat	0

CYCLE 1

1:Wash Buffer	R
1:Wash Time	0.0
1:Soak	0
1:1st Aspirate	1.0
1:1st Aspirate Trap	Hot
1:Wash Aspirate	2.0
1:Wash Aspirate Time	Cold
1:2nd Wash Aspirate Trap	Cold
1:Pause Here	Yes

This portion of the cycle is used to aspirate wash buffer to pre-wet the filter mat. An empty microplate is placed on the stage and moved up. The wash/aspirate cycle runs for 2.0 seconds which will move about 0.8ml per well through the filter mat. You may open the head following this cycle to determine if the mat is properly wetted. The wash/aspirate time can be increased or decreased accordingly. The second aspirate function merely moves the remaining liquid in the wells up through the aspirate lines.

The effluent is all directed to the cold trap. When the cycle stops, remove the empty plate and replace with the actual test plate. Pressing RUN will let the "Betty" program continue as follows:

Note: The setting of 1.0 second for the first aspirate is a precaution. It is not needed in the pre-wet cycle. However, if the operator should forget to start with an empty plate and instead places the actual test plate on the stage, the examples won't be lost. The pre-wet cycle will be lost, but the test will be saved.

CYCLE 2

2:Wash Buffer	R
2:Wash Time	0.0
2:Soak 0	
2:1st Aspirate	0.5
2:1st Aspirate Trap	Hot
2:Wash/Aspirate	1.0
2:Wash/Aspirate Trap	Hot
2:2nd Aspirate	1.0
2:2nd Aspirate Trap	Hot
2:Pause	No

This portion of the program will aspirate the sample to the filter mat, and then wash the wells with a small amount of buffer, followed with a second aspirate to dry the wells. All effluent will go to the hot trap. There is no pause, so the program continues to Cycle 3 as follows:

CYCLE 3

3:Wash Buffer	R
3:Wash Time	0.0
3:Soak	0.0
3:1st Aspirate	0
3:1st Aspirate Trap	Cod
3:Wash/Aspirate	3.0
3:Wash/Aspirate Trap	Cold
3:2nd Aspirate	3.0
3:2nd Aspirate Trap	Cold
3:Pause Here	Yes

The third cycle will wash and aspirate buffer through the wells for three seconds, followed by a three second aspirate-only cycle for drying. All effluent goes to the cold trap. The cycle stops to allow the operator to replace the now empty test plate with the small pre-wet pan. Using a squeeze bottle, add the desired amount of alcohol to the pre-wet pan and lift it to the up position. Pressing RUN push button allows the program to continue to Cycle 4 as follows:

CYCLE 4	
4:Wash Buffer	R
4:Wash Time	0.0
4:Soak	0
4:1st Aspirate	1.0
4:1st Aspirate Trap	Cold
4:Wash/Aspirate	0.0
4:Wash/Aspirate Trap	Cold (Don't Care)
4:2nd Aspirate	0.0
4:2nd Aspirate Trap	Cold (Don't Care)
4:Pause Here	No

This will aspirate a small amount of alcohol through the filter to help the drying. If more alcohol is needed to wash out the wells, switch the last two cycles to alcohol from the second wash bottle.

PRESET PROGRAMS

The program sheets at the end of this manual show five programs that have been preset by Tomtec at the time of shipment. These variables may be easily changed by the user. They are recording user-set programs. Since not all users use both the hot and cold trap, the programs are factory set to use the hot trap only.

PULSE WASH...1 (5 CYCLES)

This program uses the reagent in the right hand reagent container to pre-wet the filter and then washes the cells. An empty plate is placed on the stage for Cycle 1. The wash/aspirate function is used for two seconds to pre-wet the filter mat with approximately 1.0 ml (at 3.0psi). The empty plate is replaced with the cell plate. The cells are aspirated to the filter mat. The cells remaining in the wells are then washed

out with a series of wash/aspirates in a pulse manner. Cycle 3 is repeated five times. The wells are filled with a wash for 0.3 seconds, wetting the entire well wall. After a once second soak time, the full well contents are aspirated to the filter mat. The wash/aspirate and second aspirate is added to the cleaning function. The repeated cycles provide more activity in the well to flush the contents to the filter mat.

If the 1 ml tubes are being used instead of microplates, increase the wash time in Cycles 3, 4 and 5 to bring the liquid level higher in the tubes. We suggest doubling the 0.3 seconds used for microplates.

SLOW PULSE WASH...2 (5 CYCLES)

This program uses the reagent in the right hand container to both pre-wet the filter mat and wash the test wells. An empty plate is placed on the stage for Cycle 1. The wash/aspirate cycle 1 used for 0.5 seconds to pre-wet the filter mat. The cycle pauses to allow the operator to remove the empty plate and insert the test plate. Pressing RUN allows the cycle to continue. Cycle 2 will aspirate most of the cells to the filter mat. Cycle 3 repeated five time's long aspirate and soak times allow for sticky cells to be loosened.

SINGLE WASH...3 (2 CYCLES)

This program uses the reagent in the right hand container to both pre-wet the filter mat and wash the test wells. An empty plate is placed on the stage for Cycle 1. The wash /aspirate cycle is used for 2 seconds to pre-wet the filter mat with approximately 1.0 ml (at 3.0 psi). The empty plate is replaced with the cell plate. The cells are aspirated to the filter mat. The cells remaining in the wells are then washed out with a single wash/aspirate of six seconds.

DRY OVERFLOW...14

This program is intended to aspirate air through the overflow passageways to dry them. It would be desirable to use this program after a rinse cycle if the Harvester is going to be used immediately, rather than left idle. To use this program, it is necessary to shut off or disconnect the reagent called for (right or left) so no wash actually flows. The overflow valve will open and aspirate air through the overflow passageways.

VOLUME CHECK PROGRAM...15

Prior to each day's use, it is good practice to run a Volume Check. This program is preset at the factory, but may be changed to suit your conditions. The volume check program only operates a wash addition. The overflow is off, the wash/aspirate and aspirate are set to zero. The result creates a dispense cycle. The amount dispensed in the time set indicates the flow rate for that pressure setting.

RINSE ... 16

The Harvester is supplied with a Rinse Program as Program 16. In most cases, it will thoroughly rinse the instrument. However, since only the user has knowledge of the fluids in use, it is the user's responsibility to be sure the cleaning procedure is adequate for the solutions that were used.

To use the Rinse Program, fill one of the 9 liter carboys with the appropriate wash solution for the application. Connect it to Wash R (or whichever wash solution is set for the Rinse Program). Place an empty microplate on the stage and raise it to its uppermost position (cell harvesting position). PRESS START. The Rinse Program is set as follows. Wash solution will flow Wash R out of the wash tips into the microplate wells at the full wash pressure head. As the wells fill, the liquid level moves up to come in contact with the overflow orifices. From there, it is aspirated out through manifold to thoroughly rinse it. This action occurs for up to 25.6 seconds. Even though the overflow function is not used in the other programs, it must be washed periodically to keep it clean.

At the end of 25.6 seconds, the overflow valves automatically close, and the aspirate valves open. This wash/aspirate function now continues for another 25.6 seconds. This will move the wash solution through the aspirate manifold.

Note: The head must be closed, although a filter mat should not be in place. If extender tubes are used, then it is necessary to use the prime pan instead of a set of tubes.

In less than one minute, the system has been washed. Bear in mind the traps must be able to accommodate this liquid. If suitable trap volume is not available, the Rinse Program may be rewritten to achieve the same purpose as outlined. A pause could be incorporated to allow the manifolds – wash, overflow, and aspirate. **This is a user responsibility.**

CELL HARVESTING PARAMETERS

This section is to assist the users of the Tomtec Harvester in debugging new assay protocols. This information must be added to the user's scientific knowledge of the biology involved. A good understanding of the entire process enables the investigator to use what is known to determine what is unknown in a scientific method.

CELL HARVESTING PRINCIPALS

The Tomtec Harvester is designed to aspirate liquid borne materials through a glass fiber filter mat. The glass fibers filter the particles, such as cells, while still allowing the liquid to wash excess labeled material that was not taken up from unbound label by filtration. What is implied by the process is that the filter chosen is efficient in stopping the particle matter of interest and that sufficient wash liquid is used to rinse away the unbound label.

HARVESTER 96® APPLICATION NOTES

CELL REMOVAL FROM MICROPLATES

A Cell Harvester works on the following principles:

Room air at atmospheric pressure is sucked into a vacuum source. The speed of this air flow varies with:

a. Depth of vacuum (inches of Hg)

b. The restrictions in the pathway

By obstructing the air flow with cells suspended in a liquid, and a glass fiber filter, the cells are trapped in the filter. However, for the cells to be transported they must be in suspension in the liquid the air is moving. This note addresses some alternative actions in those cases where the cells are adherent or otherwise not in suspension.

PUTTING CELL IN SUSPENSION

There are several conventional methods of breaking away adherent cells and putting them in suspension. The individual operator is the best source of which method is most suitable and compatible with the protocol used.

AN ADDITION TO THE WASHING REAGENT

One report describes adding a small amount of EDTA, Ethylene Diamine Tetra Acetate, to the wash reagent. It was very successful for that application**.**

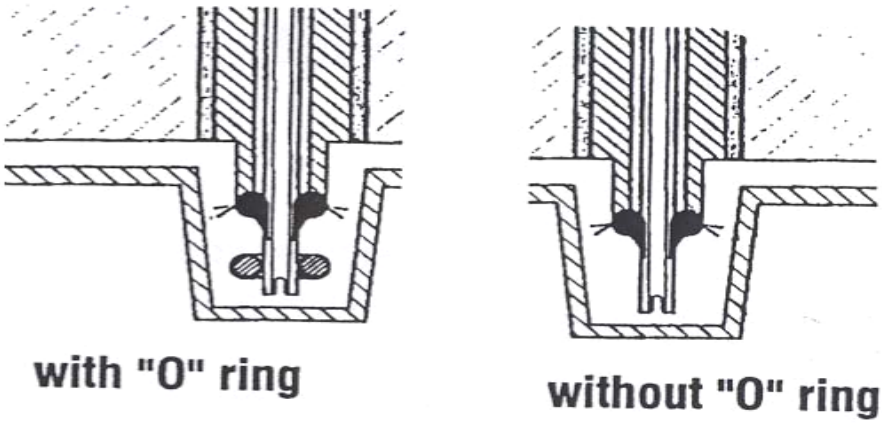
Caution: Check for compatibility.

USING THE PULSE WASH PROGRAM

The programmability of the HARVESTER 96 allows for a Pulse Wash Program. In this type of program, the well is filled and emptied several times. This increases the erosion effect by creating more action in the well. Harvesters shipped after January 1, 1993 incorporates a new Slow Pulse Program. Cycle #3 can be set to repeat the same sequence a number of times. The use is to set the wash and wash/aspirate times to just fill the well then aspirate it to empty, repeating the sequence as desired. This provides considerable action in the well to aid the removal of cells.

ADDING SMALL "O" RINGS TO THE ASPIRATE TUBES

The regular air flow is directly to the end of the aspirate tube located in the center of the well. A small "O" ring forces (Tomtec P/N 120—007-75, set of 106) can be slipped on the end of the aspirate tube. The small "O" ring forces the air flow (and wash liquid) around the outside and across the bottom of the well to the aspirate tube. This increases the erosion effect in the corner and on the bottom of the well. **(See Sketch below).**



USING CORNING EASY WASH PLATES

Corning provides a flat bottom tissue culture plate (Corning P/N 25870) that has a beveled surface at the corner instead of a sharp right angle. This plate design provides better washing action.

HAVE ALL CELLS BEEN REMOVED?

The quickest method of observing if all cells have been removed is microscopic examination. If cells are still present, run the same cycle again to determine if they can be removed by additional washing action. If so, increase the wash times **(CAUTION: Check for Flooding)** or repeat the program, which ever is most suitable.

If a MicroBeta counter is available, you may try counting what is left behind. Harvest the plate, add scintillation cocktail and count the empty plate for residual counts. Another method is to cut up the harvested (and empty) plate and put the pieces in scintillation vials. Add cocktail and count conventionally.

TELEPHONE SUPPORT FROM TOMTEC, INC.

If the results are not as desired, the following is a check list of the variables you can influence before you call us to work out a solution. Please make a few notes identifying the present settings.

CHECKLIST

Wash rate into the well: PSI setting
Wash time into the well
Vacuum during and directly after the aspirate cycle
Aspirate Time
Filter Thickness
Soak Time
Using Prewet Cycle or Not

FLOW RATES

There are two separate and independent flow rates in the Tomtec Harvester: The wash liquid rate into the well and the aspirate rate out of the well. If the wells flood, the cause is simple - wash is going in faster than it is being aspirated out.
The aspirate rate out is determined by four primary factors:

(1) Available flow rate
(2) Vacuum flow rate
(3) Resistance of the filter mat
(4) The loading of particulate matter into pores of the filter (i.e., cells).

These factors are primarily determined by the available vacuum and the assay protocol, thus there is limited freedom in changing them. The wash rate into the well is easily controlled by simply changing the head pressure.

WASH RATE IN

A precision regulator within the Tomtec Harvester regulates the air pressure from 0 to 5 psi. At 3.0, psi 0.5 ml per well per second.. At 2.0, psi 0.4 l per well per second. At 1.0, psi 0.3 ml per well per second.

It will vary with each instrument due to slight differences in the flow paths. Most cell harvesting applications are insensitive to the amount of excess wash used. There may be some exceptions, such as receptor binding assays, where the receptor is lightly held. A 20% variation in flow rates between wells is acceptable in practically all applications. If a well shows an abnormally low volume, the tip should be removed and cleaned. Low wash volume would contribute to less washing and thus higher background counts in the well.

ASPIRATE RATE OUT

The aspirate rate out of the well is not as easily adjusted as the flow rate into the well. The Tomtec Harvester is designed to harvest all 96 wells simultaneously. The 96 aspirate tubes connect each sample well in the microplate to a specific area of the filter mat. This specific area is isolated from adjacent areas by means of electrometric "O" rings on either side of the filter mat. These "O" rings compress the fibers, preventing flow laterally through the glass fiber matrix. The aspirate lines between the microplate and the filter mat are short and relatively large in diameter (.062 inch inside diameter). They provide a fast, efficient transfer from the microplate to the filter mat. We have seen no evidence of these tubes becoming clogged. They are easily checked by observing if the filter area is wet in their respective spot. A further check can be made by passing air or water through them with a syringe.

VACUUM CONSIDERATIONS

The effective open area of the 96 aspirate tubes is equivalent to a 5/8" diameter tube. This places a demand on the vacuum system for the flow rate (cubic feet per minute) more so than for vacuum head (inches of Hg). The flow rate is affected by restrictions in the flow path. The upper head of the Tomtec Harvester is accomplished by progressively increasing the available cross sectional area in the flow path as more tubes are accommodated. A large orifice solenoid valve is used to connect the trap to the upper head. A ½" ID wire reinforced, vinyl vacuum line is provided for connection to the vacuum trap and vacuum source. The trap acts as a vacuum reservoir in

addition to retaining the liquid. When the aspirate solenoid valve opens, it provides a vacuum source to quickly aspirate liquid from the microplate through the filter mat. Keep the vacuum trap line between the Harvester and the vacuum trap as short as possible. This line carries water and air. The vacuum source line from the shut-off valve to the trap can be longer since it only moves air.

Tomtec provides a special flow filter installed between the Autotrap (or vacuum flask) and the vacuum source. This filter, rated at 0.2 microns, serves two purposes. First it will trap aerosol prior to entering the vacuum source. Secondly, the hydrophobic filter medium will not pass water. Should an error allow the vacuum trap to be over filled, the water will not pass the filter. The filter will be blocked, thereby shutting off the flow. After this fault, the filter must be replaced to restore the system to its operating condition.

Use vacuum tubing with a ½" inside diameter to make the vacuum connection. Tomtec supplies seven feet of metal reinforced, vinyl tubing with each Autotrap. Additional tubing is available from Tomtec. If you are not using our Autotrap, we recommend you use at least a 4 liter side arm flask.

The Tomtec Harvester is designed to operate from house vacuum, where available. However, if the house vacuum is below 10 in. Hg, it may compromise the Harvester's operation. Further, it is essential that the standard laboratory ¼ turn vacuum cock be changed. ***See section: "Changing the Value to the House Vacuum***

If house vacuum is not available, the Harvester may be operated from a vacuum pump. The pump should have a capacity of 250-300liters/minute (8-10cubic feet per minute) at free air. At blank off, it should be able to pull 25 to 28 inches of mercury vacuum. If an oil filled vacuum pump is used, check that it is designed to handle free air. Many are designed to pull high vacuum and will overheat if handling a high volume of air. The small vacuum pumps used to operate many single row manual cell harvesters will not have enough capacity to handle the throughput of the Tomtec Harvester.

The Tomtec Autotrap has a built in vacuum gauge to monitor the vacuum conditions. House vacuum at blank off may be anywhere from 15 to 25 in. Hg. If between 20-25

in. Hg, it will probably support an incoming wash flow rate from 0.5 ml to 0.6 ml/well/sec (3.0 psi). If between 15-20 in. Hg, it may be necessary to reduce the incoming flow rate to 0.4-0.5 ml/well/sec (2.0 psi). If between 15 and 10 in Hg. the incoming flow rate may have to be reduced further (1.0 psi).

Assuming the vacuum gauge shows 20 in. Hg at blank off, the following conditions would be observed during operation. When starting, the gauge may drop to 15-18 in. Hg as it aspirates liquid. This may drop to 12-15 in. Hg as the Harvester is sucking air. With the aspirate lines wide open and no restrictions in the path, the vacuum may drop down to 5-10 in. Hg. With a filter in place, the gauge will show a higher value and provide a measure of the flow resistance of the filter.

FILTER MATS

The glass fiber mat is a filter. It is expected to trap and retain particulate matter of a certain size. If the filter pore size rated at 1 micron, it would be expected to stop the flow of most cells that were larger in size. However, it should be remembered that the pore size is obtained by the intermeshing glass fibers creating a torturous pathway that will stop some percentage of particles of various sizes. It may stop 50% of the cells at one size 75% of those that were twice as large, 90% that were three times as large, and 100% of those that were, say, five times as large.

How do you know you have chosen a filter mat with the right retention for the assay?

One method is to harvest the plate with two filter mats in the Harvester. Then count the bottom and top mat separately. The bottom mat is the one expected to retain all of the counts. If there are counts on the top mat, it implies the need for more filtration.

An argument could also be made that the counts went through the first mat because of a fast flow rate. This could be investigated by repeating exactly the same test with three filter mats, one on top of the other, instead of two. The resistance of three mats will decrease the flow rate. If the counts were higher on the first mat this time, it would implicate the flow rate. If they were comparable, then the conclusion would be finer filtration is required. Defining the problem leads to several possible solutions.

WASH FLOW

In setting up the assay protocol, the question is “How much wash should be used?” The purpose of the wash is to transfer the contents of the well to the filter mat and then wash away the unbound label. To determine if we have cleaned the well, the following test is suggested:

Harvest the plate on the normal filter mat. Then remove this first mat, and harvest the same plate with the same program on a second filter mat. If there are counts on the second mat, it indicates the well was not washed long enough to transfer all of the counts to the filter mat. Repeat the same test, but this time, run the program twice on the first filter mat. Harvest the same plate on a second filter mat to determine remaining counts.

Between programs, examine the plate microscopically to determine if there are cells left in the corners. If this is the problem, volume of wash may not be the best answer. Better agitation may be required. This can be obtained with the pulse wash program. In this program, the well is filled, allowed to soak briefly, and then aspirated, then wash/aspirated, and aspirated a second time. This cycle is repeated four or five times for the complete program.

Thus, if agitation is required instead of volume, the technique is to use the pulse wash program. This will provide the desired agitation, and the short wash/aspirate times will keep the volume of wash collected in the traps to reasonable levels.

WASH FLOW VOLUMES

As a starting point, we suggest washing the plate with 4-6 ml per well. Run the above two filter mat tests to determine if the wash is sufficient. On a number of protocols, it has been necessary to go to 12-14 ml per well to obtain low background counts. It is dependent on the type of assay being run. Try to judge whether wash volume or wash agitation is the determining factor. If it is wash volume, increase the time on the wash/aspirate cycle. If it is agitation, decrease the wash/aspirate times and increase the number of cycles.

ADHERENT CELLS

It is easy to visualize an assay where the cells are adhered to the bottom of the well so that no amount of washing will remove all of them. In this case, other methods may be investigated to release the cells. Corning has "Easy Wash" plate. This is a flat bottom with a slight incline at the corner. It eliminates the 90^{o} corner angle. The flat area is slightly smaller at 4.5mm in diameter. It may be possible to manually jiggle the plate on the Harvester stage during the harvesting cycle. The amount of movement will be limited by the wash tips.

USING SMALL WASH VOLUMES

There are times when it is desirable to transfer small volumes of other liquids through the filter mat. The dead volume in the wash delivery system can be bypassed by using the small pre-wet pan with the desired volume. Place it on the microplate stage, and bring it up in contact with the wash tips. Aspirate the contents of the pre-wet pan through the filter mat.

TROUBLE SHOOTING

The above is to provide an understanding of the cell harvesting procedure and the parameters involved. Following is an effort to tie them together in a trouble shooting sequence. The problem will be identified by inconsistent counts on what are supposed to be replicate samples. Bear in min, the readout is a measure of every step in the complete protocol from pipetting to cell growth, to harvesting, to the reader. We will only address the harvesting portion of the protocol. When a well shows counts that were not expected, the question is "Where did they come from?"

1) Is one well overflowing into an adjacent well?

   To determine if a well is overflowing, look at the interstices between the wells of the microplate after harvesting. There should be no liquid between the wells. If the Interstices are dry after harvesting, the well did not overflow. All of the wash that went into that well and its contents went to the filter mat.

2) Is there transfer between wells on the filter mat?

   Immediately after harvesting, remove the filter mat and hold it to the light. The interspaces between the "O" ring depressions should be dry while the

circle areas will appear wetted. If the space between wells on the microplate is dry and the space between the"O" rings on the filter mat is dry, it means one thing. Whatever was in the well, or added to it, only went through its designated area on the filter mat.

If the counts are lower than expected, then they may still be in the well (insufficient washing) or they may have gone through the filter mat. Tests to determine this were discussed previously.

Next

Run the same cycle with a filter mat. (The filter mat would help seal any gap between "O" rings which is why the Initial test was done without a filter mat in position to check that condition.) With a filter mat, there will be more resistance to flow, depending on the type of filter mat. If flooding occurs now, it means the incoming flow rate must be reduced accordingly.

Last

Run the actual test protocol. If flooding occurs, it probably means that the cell loading is closing up the filter mat pores, increasing its resistance to flow. The solution is to decrease the flow rate until it does not flood. With a decreased flow rate, it may be necessary to increase the wash aspirate times to put the required amount of wash through the filter mat.

CLEANING & MAINTAINING THE HARVESTER 96®

Generally, the only maintenance required is to keep the system clean. Failure to do so can cause serious damage. Such damage is not covered by our warranty. If the salts or proteins contained in many wash solutions are allowed to dry in the system, they will cake and clog the small flow passages. This problem is easily prevented by following each use period with a thorough wash procedure. However, since only the user has knowledge of the fluids in use, it is the user's responsibility to be sure the cleaning procedure is adequate for the solutions that were used.

MAINTAINING YOUR HARVESTER 96®

PREVENTING CLOGGING OF YOUR HARVESTER 96® WASH TIPS

To keep your **Tomtec Harvester 96®** in good working order, we suggest the following maintenance:

⇨ If your device is used frequently, we suggest you leave he device in a **"Wet"** state after use. In this way any residual that may be left on the wash tips will not have a chance to dry and clog the small wash orifice, to leave the **Harvester 96® "Wet"**…

→ After use, rinse through the device with the three or four liters of DI water. Be sure to use both wash inlets and trap valves, if so equipped. Then place the soak pan that is provided with each unit, onto the stage up as far as it will go. Using the **"Prime"** button, fill the pan up to the brim with DI water so that the white portions of the wash tips are fully submerged in the water. This will keep the wash tips **"Wet"** until the next use. In this way even if the entire residue is not washed out of the tips, it will not dry out and clog the small orifice. The device may be left this way for a few days. The limiting factor is how quickly the water may wash tips, drying and clogging may occur.

⇨ If your device is used infrequently or is to be placed in storage, then we suggest you leave it in a **"Dry"** state. To leave the unit in a **"Dry"** state…

→ Rinse through the device with six liters of DI water. Be sure to use both wash inlets and trap valves, is so equipped. Then repeat this step using two to four liters of 100% Methanol or Ethanol. The alcohol will displace the water in the system. Repeat once more for five minutes using empty buffer bottles. This will clear out the system of alcohol and leave it clean and dry. Again, be sure to rinse both wash inlets and trap valves, if so equipped. The unit may now be stored indefinitely.

NOTE: No filter is needed for rinsing the device. If you should have a unit that harvests to filter a filter plate, we recommend you remove the filter from the plate before rinsing the device.

Following the cleaning, we suggest leaving the Harvester wet. This will prevent any salts that might be left in the system from drying out. To do this, leave the system manifolds full of water. Use the wash function to fill the small pre-wet pan to the full up position on the microplate stage. This will submerge all of the tips, keeping them wet. To begin operation the next time, just flush the manifold with the wash solution to be used, and the Harvester is ready. Periodically, the wash tips should be brushed with a small brush such as a toothbrush.

The aspirate lines are direct and short. Should one become clogged, it is easily flushed with an air stream or syringe-applied water stream.

If the Harvester is to sit idle for a longer period of time, there is concern that growth will take place in the water. Periodically, a germicidal solution can be rinsed through it. **If a Clorox solution is used, do not leave it in the Harvester longer than necessary.**

HARVESTER 96®
TECHNICAL NOTES

CLEANING THE HARVESTER 96®

Tomtec cannot advise which chemicals are most effective in cleaning the Harvester 96 after it is used to harvest various organisms and infectious agents. This responsibility rests with each user who has knowledge of the materials being used. However, this technical note will help the user assess the effect various cleaning agents may have on the machine.

MATERIALS IN USE

Filter head assembly	Black anodized aluminum
Wash manifold	Polypropylene
Wash reagent tubing	Silicon rubber
Head Interconnect tubing	Polypropylene (Teflon on special order)
Solenoid valves	Stainless steel flash nickel plated
O-Rings	EPR and Viton
Painted parts	Aluminum with baked polyurethane

The only parts of the assembly likely to be effected during normal operation are the solenoid valves. The stainless steels parts in the valves need high iron content to work, and in the prolonged presence of many salt buffers, they will rust. Flash nickel plating of the valves minimizes the exposure, but the flash nickel layer will be removed by acids such as Trichloroacetic Acid.

CLEANING THE HARVESTER 96® USING CLOROX (Sodium Hypochlorite)

Clorox is quite corrosive. It should not be used at full strength. A 20% solution should not present a problem as long as it is not left in the Harvester 96® for any length of time. Using the rinse program, fill one of the reagent bottles with a 20% Clorox solution, and wash it through the complete system. The Harvester 96® must then be

thoroughly rinsed with water. We suggest running two (2) full reagent bottles of water through the machine following the Clorox solution. Run the rinse water through the same side (right or left) as the Clorox solution so as to wash the solenoid valve. Do not run the Clorox solution through from the other. One valve would then still have Clorox in it.

AUTOTRAP

The Autotrap will receive all the Clorox solution from this suggested rinse protocol. It should be emptied three times:

First – after the Clorox solution is used

Second – after the first wash bottle

Third – after the second wash bottle

Do not leave the Clorox solution in the Autotrap for any extended time.

The Harvester® and Autotrap should come to no harm from the use of the Clorox solution as long as the described cleaning procedure is followed.

ALGAE AND ORGANISMS

There are a number of germicidal and algaecide compounds available to avoid growth in the water/reagent bottles. The question here is more of a compatibility with the user's protocol. One compound quite commonly used in clinical chemistry is sodium azide.

TELEPHONE SUPPORT FROM TOMTEC, INC.

If you have specific problems and/or have information you would like to discuss with us, please call: 203-281-6790 (Outside USA) – Toll Free: 1-877-866-8323 (Inside USA).

Periodically, it may be desirable to rinse the Harvester with a detergent solution. Use only non-sudsing detergents; otherwise the vacuum trap will fill with suds. Laboratory glassware detergents should be suitable. Do not let the detergent solution stand in the Harvester for a period of time. Follow their use with a clean water rinse.

If the Harvester is to sit idle for an extended period of several weeks, it should be left dry.

CLEANING TIP ASSEMBLIES

The Harvester used a removable tip assembly to allow easy cleaning of the small flow passages. If the volume check program shows an abnormal well, do the following:

Slip the tip removal tool over the end of the clogged tip. Twist the knurled collar to tighten the collet on the tip. Pull the tip assembly down in a twisting motion. **(Caution: Tightening the collet too tight will squash the tip, causing the collet to grasp the metal aspirate tube).** This could remove the metal aspirate tube from the head assembly. Only the tips should be removed. If the metal aspirate tube is removed by accident, please call Tomtec for information on replacing it and connecting the tubing). The best method for cleaning the tips is to place them in an ultrasonic bath.

To replace the tip assembly, place it back on the aspirate tube. Using the tip removal tool, push it home until it seats against the stop.

SOLVENT COMPATIBILITY

The wash solution manifolds are fabricated from polypropylene. The wash tips, however, are ABS (Acrylonitrile Bbutadiene Styrene). Solvents such as acetone, ketone, ethyl acetate, or methylene chloride should not be used. Most alcohols are suitable, but the user should check for compatibility with acetal. The solenoid valve is stainless steel with a nickel pating. The main manifold is sealed anodized aluminum. The aspirate tubing is inert polyethylene with stainless steel tubing at both ends. The “O” rings and elastomeric seals are ethylene polypropylene.

Some cell harvesting protocols use a 5% or 10% solution of tri-chloro-acetic acid (TCA). TCA is commonly used to precipitate proteins. Other protocols may use a solution containing hydrochloric acid. Any time aggressive solutions such as these are used; special care must be exercised in keeping the Harvester and Autotrap clean.

These solutions should not be left in the Harvester or Autotrap any longer than necessary. Immediately following their use, run an adequate rinse cycle to remove all traces.

Even with careful rinsing after use, the user may find these aggressive liquids will shorten the life of some components. These components are not covered by the warranty. The polypropylene manifolds are specially designed to handle TCA. They will not be affected. The elastomeric seals in the solenoid valves may be affected over a period of time. The fittings on the reagent bottle and the wash tips will have a shorter than normal life. These are all relatively low cost components and are easily replaced. The usefulness of the Harvester should not be restricted to prolong the life of low cost, replaceable components. If you plan to use aggressive liquids, such as TCA, on a regular basis, you may wish to make a small investment in these spares to have on hand.

Periodically, it may be desirable to run a non-foaming detergent or germicidal solution through the Harvester. Again, **do not leave these solutions in contact with the Harvester and Autotrap for an extended period.** Follow their use with an adequate rinse cycle. If Chlorox is used as a germicidal solution, it must be a diluted solution, not full strength.

ACCESSORIES AND SPARE PARTS

The following parts are delivered with each HARVESTER 96®

5 - Spare removable wash tip assemblies
2 - Spare 1.5 amp slow-bio fuses
1 - Wash tip removal tool
10 - Spare screen plate screens
10 - Spare screen plate “O” rings
10 - Spare filter plate “O” rings
1 - 3/16 hex key for use in adjusting head

IF YOU NEED HELP

If you have any questions regarding this manual or the service and maintenance of the equipment, please contact the Tomtec service department. Also, please know the serial number of your equipment; the service person will ask you for it.

Our phone and fax number in the United States are:

Toll-Free Phone: 1-877-866-8323 (USA ONLY)

Phone: 203-281-6790 (OUTSIDE USA)

Fax: 203-248-5724

Our shipping address is:

TOMTEC INC.

1000 Sherman Avenue

Hamden, CT 06514

Website: www.tomtec.com

NOTE:

All instruments being returned for service or repair must have a return authorization number. **Please call use before shipping any equipment**. Equipment may require a completed, signed decontamination form.

HARVESTER 96®
DECONTAMINATION FORM

In order to protect personnel involved in the service and repair of instruments, one must ensure that risk factors hazardous to health (Biological-infectious material or Radioactive isotopes) are removed from the equipment in question.

This form must be signed and accompany the Tomtec instrument when it is returned for service or repair. If not, the service department has the right to refuse the instrument for service.

Model:

- ☐ Mach II S/N_____
- ☐ Mach II M S/N_____
- ☐ Mach III S/N_____
- ☐ Mach III M S/N_____
- ☐ Mach III S/N_____
- ☐ Mach IV S/N_____
- ☐ AC Autotrap S/N_____
- ☐ Autotrap 24 S/N_____

Type of Contamination:

- ☐ Biological
- ☐ Radioactive
- ☐ None

Method of Decontamination:

- ☐ Biological
- ☐ Radioactive

} Per Tomtec Technical Note TN-001

- ☐ Other (Please Specify):_____

_____

_____

_____

NOTE: In all cases, equipment must be rinsed with distilled water and emptied of all fluids, prior to returning to Tomtec.

I confirm that the instrument listed above has been submitted to an appropriate process of decontamination at this hospital/laboratory before shipped to Tomtec.

Name of Institution:_____

Institution Address:_____

Date:_____

Signature:_____

Print Name:_____

This Space Intentionally Left Blank

PROTOCOL SET UP – MACH III

HARVESTER 96® PURPOSE

This paper is a step by step guide in setting up a harvester protocol on Tomtec HARVESTER 96® Mach III Model. It is intended to augment the instructions included in the User Manual. The Mach III is designed to support the 8 x 12 format on 9 mm centers of the MICROBETA® (Wallac) and the MATRIX 96® or 9600® (Packard) instruments. For use with the MOCROBETA®, the filter mat is placed against the white guide strips are to be removed, and the filter mat is positioned by the two guide pins.

The Filter format of the Mach III has the smallest filter area of the three models of the HARVESTER 96 ® that Tomtec manufactures. For comparison, they are shown as follows:

MACH II 6 X 16 format on 15 mm centers

Filter area per well…….0.108 in^2

MACH III 8 X 12 format on 9 mm centers

Filter area per well…….0.034 in^2

MACH IV 8 X 12 format on 9 mm centers

Filter area per well…….0.426 in^2

Thus, the Mach II has three times the filter area of the Mach III, and the Mach IV has four times the filter area of the Mach II.

The small filter area of the Mach II format makes it more critical in setting the Harvester parameters. To compensate for various types of filter mat densities and cell loadings used, it is necessary to have a good understanding of the principles involved.

1.0 MAKING SURE THE HARVESTER 96® IS OPERATING PROPERLY

The Mach III should be connected as described in the User Manual. It is assumed that the programs below are those that were originally set by Tomtec as described in the User Manual.

To begin operation, proceed as follows:

1.1 Set the head pressure on the reagent containers to 3.0 psi

1.2 Prime the Harvester 96®.

1.3 Run the Volume Check cycle twice, disposing of the first. On the second run, examine the wells for uniform delivery. There will be some variation (i.e. 10-20%) between wells. There should be no abnormally low wells. If there are, take the corrective action.

This test establishes that the Harvester is delivering wash uniformly to all wells. Note, the volume in each well at 3.0 psi, the Mach III delivers approximately 0.5 ml/well/second. The volume check time is set at 0.3 seconds, thus there will be approximately 150 microliters per well.

1.4 Next, close the head without a filter mat in place and run the Pulse Wash program. Observe the microplate for any evidence of flooding. After the program stops, look at the microplate. There should be no water in the interstices between the wells. If there is, take corrective action. See the instruction manual or call Tomtec for assistance. This determines that the Harvester is set up properly and is working.

1.5 Simulate the filter mat with a double layer of paper toweling, preferably brown toweling. Then close the clamp. Repeat the Pulse Wash program. Again, look for any evidence of flooding in the Microplate. At the conclusion of the program, hold the filter mat to the light and examine for cross flow between

wells. There should be wet circles and dry corner quadrants. This test indicates that the filter head "O" rings are sealing off each well.

The volume check test in 1.3 above demonstrates that liquid is being added uniformly to all wells. If the wells did not overflow (i.e., no flooding), then this uniform volume had to pass through the filter mat. The filter test just completed shows there was no cross flow. Thus, whatever was in the well had to pass through its respective filter area?

A Word of Caution – resist the temptation to tighten the filter head clamp to correct a perceived problem. There are probably 99 other solutions that have a better chance of success. Over tightening the head can cause many more problems than it cures. If in doubt as to how to solve a problem, PLEASE CALL TOMTEC. We can help.

2.0 SETTING THE WASH FLOW RATE

The above tests should be used to determine that the Harvester is working properly. The following procedures are to set the parameters for the protocol. In any harvester, there are two separate flow rates – the wash rate into the well and the aspirate rate out. If the plate floods, it is due to only one reason – liquid is going in faster than it is going out. The aspirate rate is determined by the available vacuum, the resistance of the filter mat, and the cell loading. These factors are generally fixed by the protocol. The wash rate in is controlled by o.5ml/well/second. At 2.0 psi, it is 0.4 ml/well/second. At 1.0 psi, it is 0.3 ml/well/second. The user manual describes how to determine the exact rate for your instrument.

2.1 Place the filter mat to be used in the head, and run the Pulse Wash program with an empty plate. Look for evidence of flooding. This test determines what flow rate can go through the filter mat with no cell loading.

2.2 Next, determine the flow rate with the required cell loading. Using the Pulse Wash program, harvest a test plate of the cell loading to be used. Preferably, these should be "cold" cells, without radioactivity, but of the concentration to be the used in the protocol.

Look for evidence of flooding. If the wells start to flood, press STOP to stop the cycle. Lower the air pressure from 3.0 psi to a lower value to decrease the incoming wash rate. Then repeat the test. Do not go below 1.0 psi. At pressures lower than 1.0 psi, the wash manifold will lose uniformity in delivery.

3.0 SETTING PROGRAM PARAMETERS

Assume that the pressure has been lowered to 1.0 psi, and flooding still occurs. There are other parameters that can be changed to correct the problem.

3.1 First, watch the vacuum gauge on the Autotrap. If the overflow is selected as "active" it may be dumping the vacuum following the "Wash" in Cycles 3, 4 and 5. Then, the first aspirate and wash/aspirate cycles start before the vacuum has recovered. The solution is to increase the soak times to 2 or 3 seconds to allow the vacuum to rebuild in the Autotrap. Watch the vacuum gauge during the first and second aspirate times. They may be too long and dropping the vacuum excessively.

3.2 When setting the first aspirate time, it should be long enough to aspirate all of the liquid from the wells following each addition of wash. If the time set is too short, there will be residual wash left in the wells as the start of the wash/aspirate portion of cycle. However, an excessive air, rather than water and air.

3.3 The same applies to the second aspirate time. It must be long enough to remove any residual water left in the well after the wash/aspirate, but not so long as to drop the vacuum excessively.

3.4 Assume the first and second aspirate times are set properly so as to remove the residual water and not impact the available vacuum, but plate flooding persists. The next parameter to be adjusted is the wash/aspirate time. It can be reduced to the lower limiting point whereby it is only on long enough to fill the well (i.e., deliver only 300 microliters).

4.0 REMOVING CELLS AND BACKGROUND COUNTS

The above procedure has now set the parameters so that the wash rate into the well never exceeds the aspirate rate out, and there is no plate flooding. The liquid that was put into the well went through the filter mat. The next text is to determine if the cells have been removed from the wells and if enough wash has been put through the filter mat to remove the unbound material.

The repetitive filling and aspirating of the wells, provided by the Pulse Wash cycle aids in cleaning the cells from the bottom of the plate. For a harvester to work, the cells must be in corners, particularly of a flat bottom plate, may be difficult to clean.

4.1 After arriving at acceptable parameters, as described above, harvest the plate using the Pulse Wash program (5 cycles). Do not remove the filter mat yet, but do remove the microplate. Examine it under a microscope to determine if cells are still remaining in the wells. If cells are left in the plate, harvest it again onto the same filter mat using the Pulse Wash program (Press the RUN button. The first pre-wet cycle will just be another wash cycle). Again, look at the plate for cells. The objective is to determine how many times to run the program to achieve an acceptable transfer of cells to the filter mat.

4.2 It may be that repetitive running of the Pulse Wash program is desirable to clean the cells from the wells. However, this may pass an undesirable amount of wash to the waste traps. If so, the wash/aspirate times per cycle can be reduced to control the overall amount of wash consumed.

5.0 FILTER MAT SELECTION

Another variable to be examined in setting up a protocol is the filter mat itself. The filter mat is just what its name implies. It is a filter designed to trap particulate matter (cells) by providing a labyrinth pathway through which the particles cannot pass.

The simplest test is to harvest the test plate with two filter mats, one on top of the other. If there are counts on the top mat, they went through the bottom mat and would otherwise be lost.

6.0 NEED ASSISTANCE?

If you have reviewed all of the above and are still having problems, PLEASE CALL US. We can help. Our phone and fax numbers in the USA are as follows:

PHONE: **203-281-6790 9 (Outside USA) or (1-877-TOMTEC3) (Toll Free USA Only)**

FAX: **203-248-5724**

WEBSITE: **www.tomtec.com**

MACH III FLOODING PROBLEMS

The Harvester 96, Mach III, has about ¼ of the filter area per well of the Mach II. This is due to the 8 X 12 spacing on 9 mm centers of the MICROBETA compared to the 6 X 16 spacing on 15 mm centers of the BETAPLATE. As particulate matter, cells or protein, are loaded onto this small filter area, the flow rate through it decreases. Unless the incoming wash rate is reduced, the microplate will flood during the wash/aspirate portion of the cycle.

RECOMMENDATION

Reduce the head pressure on the reagent bottles by adjusting the pressure regulator on the back of the Harvester. The following are approximate wash rates:

3.0 psi	0.5 ml/well/sec
2.0 psi	0.4 ml/well/sec
1.0 psi	0.3 ml/well/sec

We do not recommend head pressure of less than 1.0 psi as this may cause uneven flow rates across the plate.

If flooding persists at 1.0 psi, the aspirate rate is still less than the minimum wash rate. The solution is to reduce the wash and wash/aspirate time so that the liquid delivered to the well does not exceed the fill volume. Then set the aspirate times to empty the well before the next filling.

PROCEDURE

To obtain optimum uniformity of the wash addition, we suggest that the head pressure be reset to 3.0 psi. Set the wash and wash/aspirate times just long enough to fill the well, i.e. 0.3 to 0.5 seconds. Increase the first and second aspirate times long enough to empty the well prior to the next filling. One potential drawback of this mode of operation is that the total wash volume is reduced and may leave the background count too high.

However, the HARVESTER 96® allows for a Pulse Wash program. In this type of program, the well is filled and emptied several times in quick succession. Harvesters shipped after January 1, 1993 incorporate new software (version 1.6x) and a Slow Pulse Wash program. (Please contact us or Wallac for an upgrade if you believe this will improve your results). In this new software, cycle #3 can be set to repeat the same protocol a number of times. Use it to set the wash and wash/aspirate times to just fill the well and then set the aspirate time long enough to completely empty the wells. The multiple wash/aspirate cycles creates agitation helpful in dislodging cells from the wells.

HARVESTER 96®
TECHNICAL NOTES

Decontamination of the Harvester 96®

CAUTION: ALWAYS WEAR RUBBER GLOVES WHEN SERVICING

THE HARVESTER 96® AND AUTOTRAP.

HARVESTER MODELS: MACH II, MACH III, & MACH IV

☐ **Biological Use:**

1. Wash the filter plate and screen plate assemblies with a bleach such as Clorox containing at least 5.25% sodium hypochlorite and rinse with distilled water.

   CAUTION: DO NOT GET THE FRONT PANEL WET

2. Prepare a solution consisting of 20% bleach and 80% distilled water.
3. Select program “Rinse 16”; set the following parameters:
   Overflow activity…….yes
   Wash time……..15 seconds
   Wash/aspirate……25 seconds
4. Run program “Rinse 16” for 2 cycles using the bleach solution and 3 cycles with distilled water as a rinse using the same wash inlet.
5. Using an empty reagent container, run the Pulse Wash program to remove all fluids from the Harvester.
6. Drain and rinse the Autotrap with distilled water to remove the bleach solution from the valves.

☐ **Radioactive Use:**

1. Follow the above procedure using a decontamination solution (i.e., COUNT OFF, DECON, etc.) per manufacturers recommendations in place of the bleach solution.
2. Verify equipment has safe level of background counts.

HARVESTER MODELS: MACH II M, MACH III M

☐ Biological Use:

1. Wash the filter and screen plate assemblies with a bleach such as Clorox containing at least 5.25% sodium hypochlorite and rinse with distilled water.

CAUTION: DO NOT GET THE FRONT PANEL WET.

2. Prepare a solution consisting of 20% bleach and 80% distilled water.
3. Run the Harvester for 30 seconds using the bleach solution and 45 seconds with distilled water as a rinse as holding the Wash/Aspirate button for the aspirate time.
4. Using an empty reagent container, hold down the Wash/Aspirate button to remove all fluids from the Harvester.
5. Drain and rinse the Autotrap with distilled water to remove the bleach solution from the valves.

☐ Radioactive Use:

1. Follow the above procedure using a decontamination solution (i.e. COUNT OFF, DECON, etc.) per manufacturers recommendations in place of the bleach solution.
2. Verify equipment has safe level of background counts.

AUTOTRAPS

☐ Biological Use:

1. Fill the Autotrap with a solution containing 20% bleach mixed with distilled water.
2. Drain and rinse with distilled water to remove the bleach solution from the valves.

☐ Radioactive Use:

1. Follow the above procedure using a decontamination solution (i.e. COUNT OFF, DECON, etc.) Per manufacturers recommendations in place of the bleach solution.
2. Verify equipment has safe level of background counts.

HARVESTER 96® PROGRAM SHEET

Program Name: Pulse Wash 1

Number of Cycles: 5 **Creator**: Tomtec Inc. **Date:** 1/9/93

Operator Functions: Place empty plate on stage at start. Replace with test plate when cycle pauses. Remove filter mat when cycle stops.

Pressure Setting: 3.0 Max Reagent Container to be used: R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	10				
Dry Trap:	Hot				
Overflow Active:	No				
Cycle 3 Repeat:			5		
Wash Buffer:	R	R	R	R	R
Wash Time:	0.0	0.0	0.3	0.3	0.3
Soak Time:	0.0	0.0	1.0	1.0	1.0
1st Aspirate Time:	1.0	1.0	1.0	1.0	1.0
1st Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Wash/Aspirate Time:	2.0	2.0	2.0	2.0	2.0
Wash/Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
2nd Aspirate Time:	1.0	1.0	1.0	1.0	5.0
2nd Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Pause Here:	Yes	No	No	No	No

Note: Values set to zero are bypassed in the cycle.

Program Notes: The above program assumes only the Hot Trap is used.

HARVESTER 96® PROGRAM SHEET

Program Name: Slow Pulse Wash 2 **Date:** 1/9/93

Number of Cycles: 5 **Creator**: Tomtec Inc.

Operator Functions: Place empty plate on stage at start. Replace with test plate when cycle pauses. Remove filter mat when cycle stops.

Pressure Setting: 3.0 psi **Reagent Container to be used:** R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	10				
Dry Trap:	Hot				
Overflow Active:	Yes				
Cycle 3 Repeat:			5		
Wash Buffer:	R	R	R	R	R
Wash Time:	0.0	0.0	0.3	0.3	0.3
Soak Time:	0.0	0.0	1.0	1.0	1.0
1st Aspirate Time:	1.0	1.0	1.0	1.0	1.0
1st Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Wash/Aspirate Time:	0.5	2.0	2.0	2.0	2.0
Wash/Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
2nd Aspirate Time:	3.0	1.0	1.0	1.0	5.0
2nd Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Pause Here:	Yes	No	No	No	No

Note: Value set to Zero are bypassed in the cycle.

HARVESTER 96® PROGRAM SHEET

Program Name: Single Wash 3 **Date:** 1/9/93

Number of Cycles: 5 **Creator**: Tomtec Inc.

Operator Functions: Place empty plate on stage at start. Replace with test plate when cycle pauses. Open head when cycle beeps. Remove filter mat when cycle stops.

Pressure Setting: 3.0 psi **Reagent Container to be used:** R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	0				
Dry Trap:	Hot				
Overflow Active:	Yes				
Cycle 3 Repeat:					
Wash Buffer:	R	R	R	R	R
Wash Time:	0.0	0.0	0.0	0.0	0.0
Soak Time:	0.0	0.0	0.0	0.0	0.0
1st Aspirate Time:	1.0	1.0	0.0	0.0	0.0
1st Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Wash/Aspirate Time:	2.0	6.0	0.0	0.0	0.0
Wash/Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
2nd Aspirate Time:	1.0	5.0	0.0	0.0	0.0
2nd Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Pause Here:	Yes	No	No	No	No

Note: Value set to Zero are bypassed in the cycle.

Program Notes: The above program assumes only the Hot Trap is used.

HARVESTER 96® PROGRAM SHEET

Program Name: Cold Rinse PGM 13 **Date:** 1/7/15

Number of Cycles: 1 **Creator**: Tomtec Inc.
Operator Functions: Same as program 16

Pressure Setting: 3.0 Max **Reagent Container to be used:** R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	0.0				
Dry Trap:	Cold				
Overflow Active:	Yes				
Cycle 3 Repeat:					
Wash Buffer:	R	R	R	R	R
Wash Time:	0.7	0.0	0.0	0.0	0.0
Soak Time:	4.0	0.0	0.0	0.0	0.0
1st Aspirate Time:	6.0	0.0	0.0	0.0	0.0
1st Aspirate Trap:	Cold	Cold	Cold	Cold	Cold
Wash/Aspirate Time:	25.4	0.0	0.0	0.0	0.0
Wash/Aspirate Trap:	Cold	Cold	Cold	Cold	Cold
2nd Aspirate Time:	5.0	5.0	0.0	0.0	0.0
2nd Aspirate Trap:	Cold	Cold	Cold	Cold	Cold
Pause Here:	No	No	No	No	No

Note: Value set to Zero are bypassed in the cycle.

HARVESTER 96® PROGRAM SHEET

Program Name: Dry Overflow 14 **Date:** 1/9/93

Number of Cycles: 1 **Creator**: Tomtec Inc.
Operator Functions: Disconnect or shut off the left reagent so no wash can flow

Pressure Setting: 3.0 Max **Reagent Container to be used:** R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	0.0				
Dry Trap:	Hot				
Overflow Active:	Yes				
Cycle 3 Repeat:					
Wash Buffer:	L	R	R	R	R
Wash Time:	10.0	0.0	0.0	0.0	0.0
Soak Time:	0.0	0.0	0.0	0.0	0.0
1st Aspirate Time:	1.0	1.0	0.0	0.0	0.0
1st Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Wash/Aspirate Time:	0.0	0.0	0.0	0.0	0.0
Wash/Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
2nd Aspirate Time:	1.0	5.0	0.0	0.0	0.0
2nd Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Pause Here:	No	No	No	No	No

Note: Value set to Zero are bypassed in the cycle.

HARVESTER 96® PROGRAM SHEET

Program Name: Volume Check 15 **Date:** 1/9/93

Number of Cycles: 1 **Creator**: Tomtec Inc.

Operator Functions: Place empty microplate on the stage to catch the contents of one dispense cycle.

Pressure Setting: 3.0 **Reagent Container to be used:** R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	0.0				
Dry Trap:	Hot				
Overflow Active:	Yes				
Cycle 3 Repeat:					
Wash Buffer:	R	R	R	R	R
Wash Time:	0.3	0.0	0.0	0.0	0.0
Soak Time:	0.0	0.0	0.0	0.0	0.0
1st Aspirate Time:	0.0	0.0	0.0	0.0	0.0
1st Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Wash/Aspirate Time:	0.0	0.0	0.0	0.0	0.0
Wash/Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
2nd Aspirate Time:	0.0	0.0	0.0	0.0	0.0
2nd Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Pause Here:	No	No	No	No	No

Note: Value set to Zero are bypassed in the cycle.

HARVESTER 96® PROGRAM SHEET

Program Name: Hot Rinse 16 **Date:** 1/9/93

Number of Cycles: 1 **Creator**: Tomtec Inc.

Operator Functions: Place empty microplate on the stage in the "Up" position. Close filter head without a filter mat in place or, use a filter plate with filter media removed.

Pressure Setting: 3.0 Max **Reagent Container to be used:** R

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	0.0				
Dry Trap:	Hot				
Overflow Active:	Yes				
Cycle 3 Repeat:					
Wash Buffer:	L	L	L	L	L
Wash Time:	0.7	0.0	0.0	0.0	0.0
Soak Time:	0.4	0.0	0.0	0.0	0.0
1st Aspirate Time:	0.6	1.0	0.0	0.0	0.0
1st Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Wash/Aspirate Time:	25.4	0.0	0.0	0.0	0.0
Wash/Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
2nd Aspirate Time:	5.0	0.0	0.0	0.0	0.0
2nd Aspirate Trap:	Hot	Hot	Hot	Hot	Hot
Pause Here:	No	No	No	No	No

Note: Value set to Zero are bypassed in the cycle.

Program Notes: If the HARVESTER 96® is us set up with extender tubes, use the prime pan instead of a rack of tubes...

HARVESTER 96® PROGRAM SHEET

Program Name:_____ **Date:** _____

Number of Cycles: ____ **Creator**: Operator

Functions:_____
_____
_____
_____

Pressure Setting: _____ **Reagent Container to be used:** _____

Program Settings:	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Dry Time:	_____	_____	_____	_____	_____
Dry Trap:	_____	_____	_____	_____	_____
Overflow Active:	_____	_____	_____	_____	_____
Cycle 3 Repeat:	_____	_____	_____	_____	_____
Wash Buffer:	_____	_____	_____	_____	_____
Wash Time:	_____	_____	_____	_____	_____
Soak Time:	_____	_____	_____	_____	_____
1st Aspirate Time:	_____	_____	_____	_____	_____
1st Aspirate Trap:	_____	_____	_____	_____	_____
Wash/Aspirate Time:	_____	_____	_____	_____	_____
Wash/Aspirate Trap:	_____	_____	_____	_____	_____
2nd Aspirate Time:	_____	_____	_____	_____	_____
2nd Aspirate Trap:	_____	_____	_____	_____	_____
Pause Here:	_____	_____	_____	_____	_____

Note: Value set to Zero are bypassed in the cycle.

HEAD CLAMPING PRESSURE ADJUSTMENT

TOOLS NEEDED

Feeler Gauge
3/16 Hex Key (supplied with each unit)
2 Phillips Screwdriver
Rubber Gloves

WARNING: Before servicing the equipment, always follow the "Decontamination" instructions on Page 48.

PROCEDURE:

Step 1: Inspection

With the head clamped down, check that the springs on the Locking Plate Assembly are not fully compressed and that they appear to be compressed equally. **(See figure19)** Be sure to check both the springs on the Right and left side plates.

If these conditions are satisfactory, proceed to Step 3.

If these conditions are not satisfactory, Proceed to Step 2.

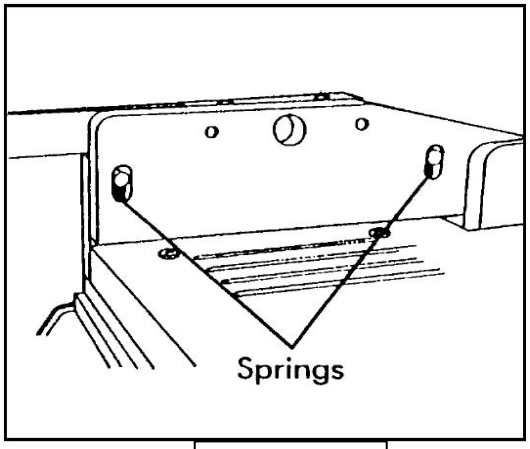


Fig. 19

Step 2: Spring Compression Adjustment

a) Unclamp the Head, and remove the (4) Screws on the Head Screen Plate. Then remove the plate. **(See figure 20)**

b) The removal of the Head Screen Plate will reveal the underside of Locking Plate Assembly. Notice the stepped holes of the Spring Adjusting Screws. Two of these screws will be on the right side plate and (2) on the left. Back out the Spring Adjusting Screw just beyond the step in the hole.

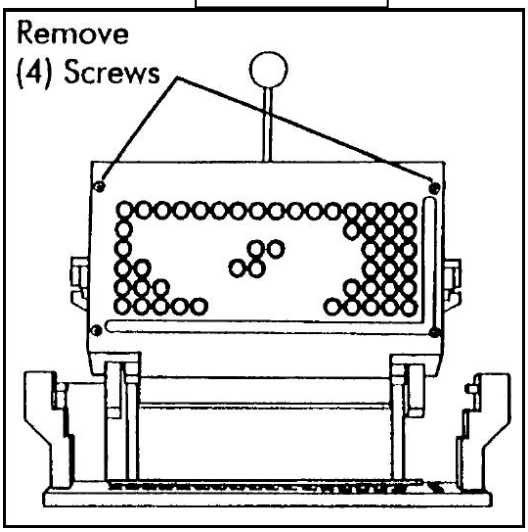


Fig. 20

c) Next, slowly turn the screws back in until the back of the screw is even with the step. Then make (4) more complete clockwise turns.

d) Replace the Head Screen Plate. Clamp down the Head, and check again that the springs are not full compression (wire-to-wire).

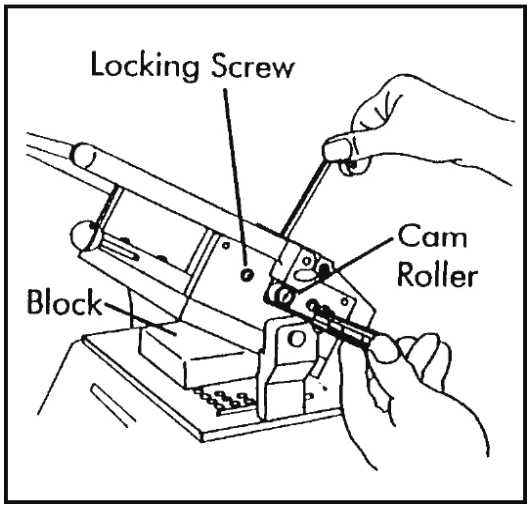


Fig. 21

Step 3: Setting the Gap

a) Prop up the Head Screen Plate Assembly with a block, (see Figure 21). Pull the Handle down into its locked position.

b) Loosen the locking screw on both sides of the Cam Roller. This will allow the Cam Roller to be adjusted.

NOTE: Be certain that the Handle is down in its locked position before processing.

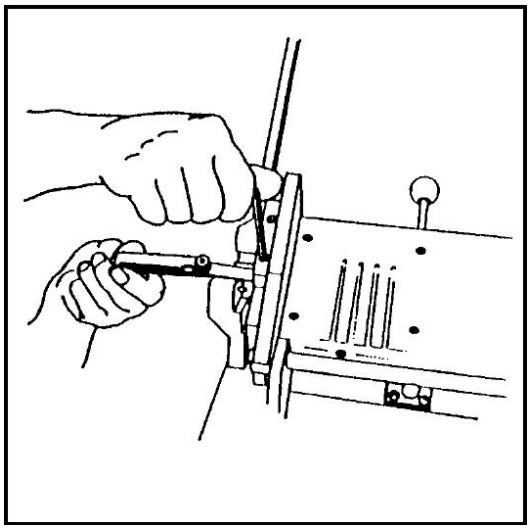


Fig. 22

c) Insert the 3/16 inch Hex Key through the holes on the top of the Locking Plate Assembly. (See Figure 22) and into the socket head screws.

Turning the Socket Head Screws clockwise will increase the gap and result in increased clamping pressure.

Turning the Socket Head Screws counter-clockwise will decrease the gap and result in decrease clamping pressure.

Use a feeler gauge inserted between the bottom of the Cam Roller and the bottom of the opening in the side plate to set the gap.

d) Repeat these steps on the Locking Plate Assembly on the other side. Use the feeler gauge to set the gap so that both sides are the same.

NOTE: A few thousandths of an inch difference in the gap can make an appreciable difference in the clamping pressure.

e) Re-tighten the four (4) Locking Screws. Check that the Locking Screws are similarly positioned in each of their slots. This can be determined by visually comparing the size of the openings under the Locking Screws. They must be the same in order to assure that equal pres-sure is being applied to both the front and the rear of the plate.

Step 4: Pressure of Head

If possible, test the closing pressure of the head with a spring tension gauge (i.e. fish scale). Use a filter mat (or a substitute) in position between the plates.

- Mach II & IV 14 to 20 lbs.
- Mach III 10 to 14 lbs.