SAKURA

Tissue-Tek® VIP™

Vacuum Infiltration Processor

Operating Manual

E150/E300 Series

SAKURA

Manufactured for:
Sakura Finetechnical Co., Ltd., Tokyo, 103, Japan
Sakura Finetek U.S.A., Inc., Torrance, CA 90504 USA
Sakura Finetek Europe B.V., Zoeterwoude, Netherlands
Made in U.S.A.

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TABLE OF CONTENTS

CAUTION AND WARNING LABELS

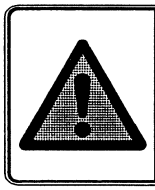
A

WARNING! PRECAUTIONS!

MAY CONTAIN FLAMMABLE LIQUIDS.
KEEP OPEN FLAMES AND IGNITION
SOURCES AWAY.

PEUT CONTENIR DES LIQUIDES INFLAMMABLES.
TENIR ELOIGNER DES FLAMMES ET
AUTRES SOURCES DE CHALEUR.

B



WARNING:
HOT SURFACES

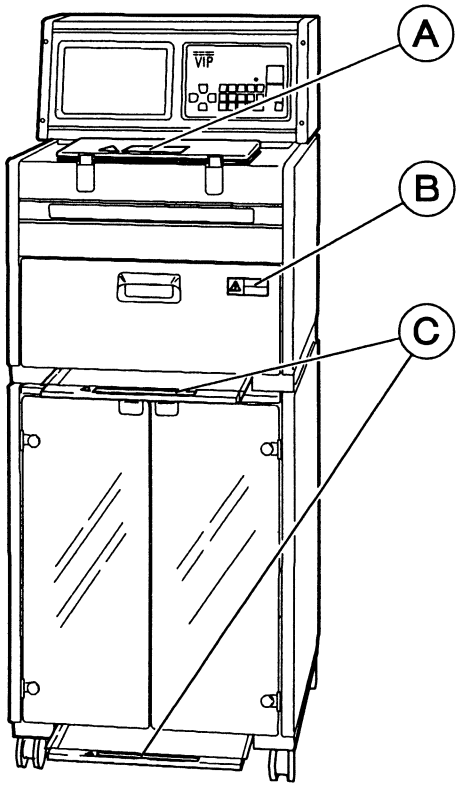
AVERTISSEMENT:
SURFACES BRÛLANTES

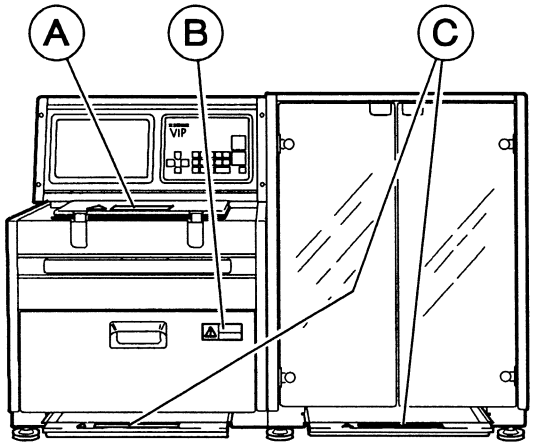
C

CAUTION! AVERTISSEMENT!

MAY CONTAIN FLAMMABLE LIQUID.
PEUT CONTENIR DU LIQUIDE INFLAMMABLE.

CAUTION AND WARNING LABEL LOCATIONS





INTRODUCTION

General Description

The Tissue-Tek® VIP™ Vacuum Infiltration Processor is an automatic, self-contained tissue processor. It is available as Model E150, which holds up to 150 tissue samples, and as Model E300, which holds up to 300 samples. Both sizes are available as a floor model (Figure 1-1) and benchtop model (Figure 1-2).

The VIP software is programmable for up to nine different programs for use in the fixation, dehydration, clearing, and paraffin infiltration of a variety of human, animal, or plant tissue specimens. The operator can program the instrument to begin processing either immediately or in a delay mode. In delay mode, the operator enters a desired end time and the instrument then automatically calculates the program start time.

Figure 1-1

The instrument consists of four major components:

- the control panel, through which all operations are controlled
- the retort, an enclosed chamber that holds the tissue specimens and in which all processing occurs
- the paraffin oven, which maintains molten paraffin at the appropriate temperature for use in the infiltration step
- the reagent cabinet, which holds the bottles of reagents.

The operator places baskets containing tissue embedding cassettes into the retort. The instrument then sequentially moves processing reagents and molten paraffin into and out of the retort through the use of vacuum and pressure. The closed-system design along with the filtering and ventilation system, ensure a sealed, moist environment for the tissues and an essentially fume-free environment outside the VIP.

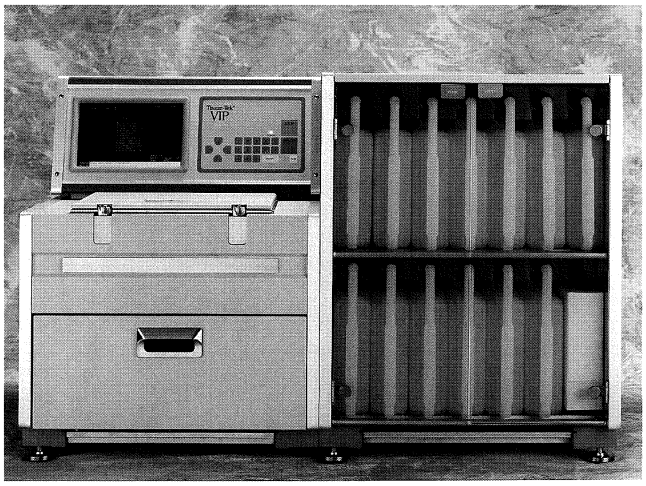


Figure 1-2

Physical Characteristics

NOTE: All figures in this manual use the floor model. Its components are identical to those of the bench model, except that the module that holds the reagent cabinet is located to the right side of the retort and control panel, rather than beneath.

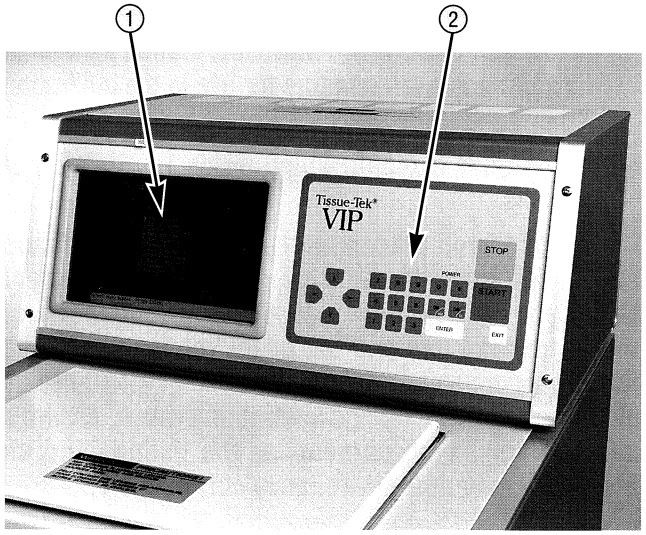


Figure 1-3

Control Panel (Figure 1-3)

The control panel contains the screen display ① and the keypad ②, which the operator uses to communicate with the instrument software.

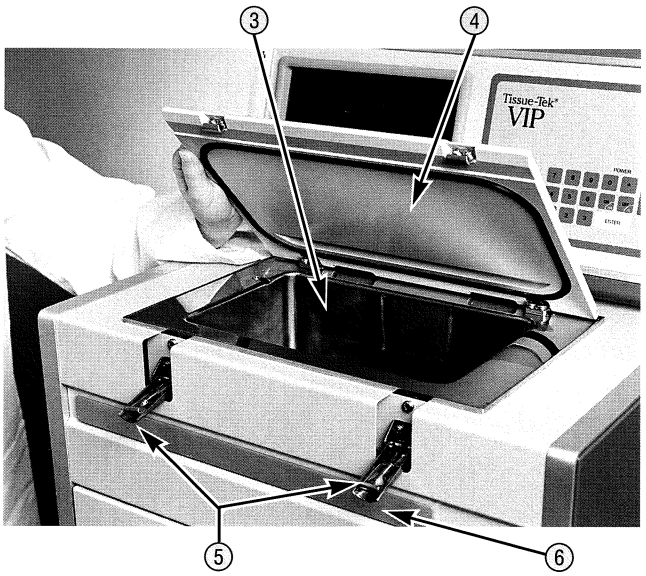


Figure 1-4

Retort (Figure 1-4)

The retort ③ is the chamber in which processing actually occurs. One or more baskets, which hold the specimens, are placed into the retort. During processing the retort lid ④ is held securely closed by two adjustable latches ⑤. The retort interlock slide bar ⑥, when in place over the latches, prevents access to the retort. During processing, when the retort bar is moved to the open position, processing stops immediately, and an alarm sounds until the retort bar is moved to the closed position.

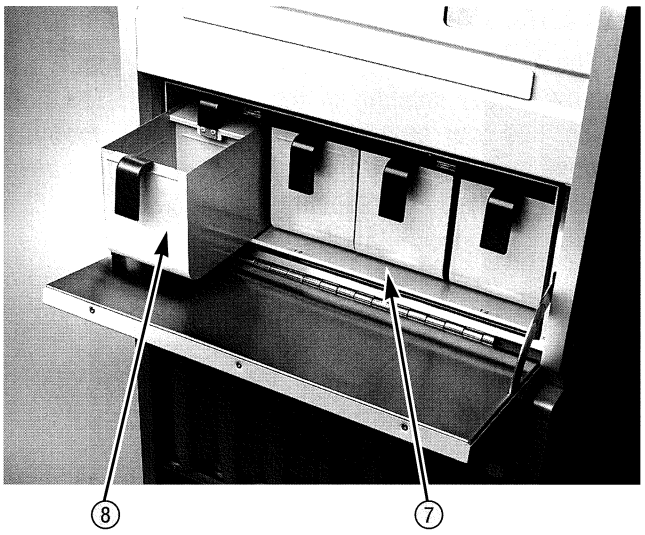


Figure 1-5

Paraffin Oven (Figure 1-5)

The paraffin oven ⑦ maintains a set temperature to hold molten paraffin at the proper temperature for use in the infiltration step. The oven holds four paraffin containers ⑧ that are easily removed by the operator for refilling. These containers are designated as stations 11 through 14. A spill tray is located under the oven to catch any spills from the paraffin containers.

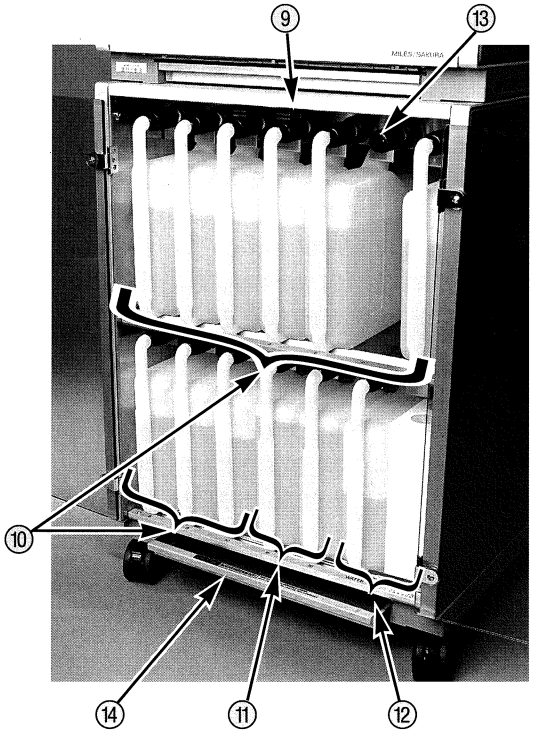


Figure 1-6

Reagent Cabinet (Figure 1-6)

The reagent cabinet ⑨ holds ten bottles of reagents, designated as stations 1 through 10 ⑩. Two additional stations (15 and 16) hold bottles for xylene and alcohol ⑪, used during the cleaning cycle. The final two spaces hold a bottle for water and the activated carbon cartridge ⑫, which are the essential components of the fume control system of the instrument. Each bottle connects into the instrument through a quick-release connector ⑬. A second spill tray ⑭ is located under the reagent cabinet to catch any spills from the reagent bottles.

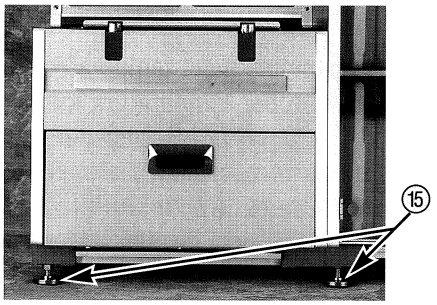


Figure 1-7

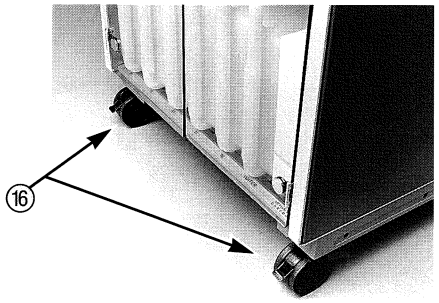


Figure 1-8

Leveling Feet/Casters (Figures 1-7 and 1-8)

The bench model has six adjustable feet ⑮ to ensure proper leveling of the instrument. The floor model has four casters ⑯ for instrument mobility; positive locks on the front casters hold the instrument securely in place for stationary use.

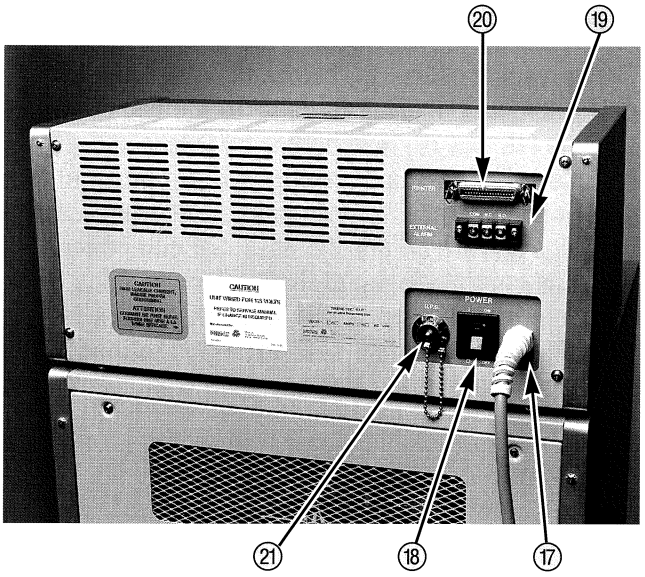


Figure 1-9

Rear of the Instrument (Figure 1-9)

The line cord ⑰ is permanently attached to the instrument. The other end of the cord attaches to an appropriate AC electrical outlet. The instrument is turned *on* and *off* with the power switch ⑱. The panel also contains a connector for an external alarm ⑲. A power outage will activate the external alarm immediately.

The following conditions will activate the external alarm five minutes after they occur: the retort bar remains in the open position during any operation (including a DELAY cycle); a condition leading to an error or warning display is left unattended; or power has been restored following an outage.

The rear panel can accommodate optional interfaces for a printer and for an uninterruptible power supply (UPS). With the printer option ⑳ installed, most any parallel printer can be interfaced to provide printouts. The UPS option ㉑ allows the instrument to be connected to a backup generator. Contact your Tissue-Tek instrument representative for information on the retrofit kits for these two options.

Principles of Operation

When specimen processing begins, the rotary valve in the instrument opens the passage to the retort from the first reagent station for which a processing time is programmed. A vacuum is created in the retort, and it draws the reagent from the bottle into the retort. This action is called a "pump-in." The rotary valve then closes, and the specimens are immersed in the reagent for the selected length of time. After this time, pressure is created in the retort and the rotary valve opens again, forcing the reagent from the retort back into the reagent bottle. This action is called a "pump-out." The rotary valve then rotates to the next station for which a processing time is programmed and the procedure is repeated.

During the immersion time, three additional options are available as histologic choices for the treatment of the specimens. Application of any of these three physical conditions can increase the reagent infiltration of the specimens. These three options may be programmed into individual and/or all stations.

P/V Cycle: The instrument alternates applying pressure and vacuum to the retort.
Agitation: A pump-out and pump-in of the same reagent is repeated every 20 minutes.
Heat: The surfaces of the retort are heated to the programmed temperature for the duration of the immersion time for that station.

After completion of a processing run, the instrument automatically prompts for a cleaning cycle. This can be overridden ONLY if no reagents from Stations 11-14 (paraffin) were pumped into the retort. In the Clean Cycle, the retort heats and the remaining melted paraffin is pumped into the last paraffin station for which a processing time was programmed. Xylene is then repeatedly pumped into and out of the retort. This is followed by multiple rinses with alcohol.

Safety Features

- The VIP instrument is designed as a closed-system tissue processor to minimize the release of reagent fumes into the environment. It also contains a fume control system, consisting of water and an activated carbon filter, to purify the air that it releases.
- The retort slide bar prevents access to the retort during processing. If the bar is slid to the left (open), processing stops immediately, any vacuum/pressure in the retort is released, and the audible alarm sounds.
- If the instrument is unable to pump a reagent into the retort (e.g., the bottle is empty or not properly connected), the reagent from the previous station will be pumped back into the retort and the audible alarm sounds.
- The instrument contains an alarm that sounds whenever there is an error condition that stops processing. The instrument can also be connected to an external alarm that will alert personnel in case of an error during the night or weekend when the laboratory is not occupied.
- A password can be entered into the software to prevent access to the program settings and processing function by unauthorized personnel.

Specifications

Power Required:

Model Numbers:
- 4890 — Bench, E150
- 4892 — Floor, E150
- 4894 — Bench, E300
- 4896 — Floor, E300

115 VAC, ± 10%, 60 Hz, 10.5 A

Model Numbers:
- 4886 — Bench, E150
- 4887 — Floor, E150
- 4888 — Bench, E300
- 4889 — Floor, E300

100 VAC, ± 10%, 50/60 Hz, 9.5 A

Model Numbers:
- 4891 — Bench, E150
- 4893 — Floor, E150
- 4895 — Bench, E300
- 4897 — Floor, E300

220 VAC, ± 10%, 50 Hz, 4.7 A
240 VAC, ± 10%, 50 Hz, 5.0 A

Dimensions:

Models E150:

Floor Model:
- Depth — 58.0 cm (22.8 in.)
- Width — 50.0 cm (19.7 in.)
- Height — 130.5 cm (51.2 in.)

Bench Model:
- Depth — 58.0 cm (22.8 in.)
- Width — 100.0 cm (39.4 in.)
- Height — 66.9 cm (26.0 in.)

Models E300:

Floor Model:
- Depth — 58.0 cm (22.8 in.)
- Width — 50.0 cm (19.7 in.)
- Height — 134.0 cm (52.8 in.)

Bench Model:
- Depth — 58.0 cm (22.8 in.)
- Width — 100.0 cm (39.4 in.)
- Height — 66.9 cm (26.0 in.)

Weight:

Models E150 — Approximately 124 kg (273 lb.)
Models E300 — Approximately 126 kg (278 lb.)

Capacity/Fill Volumes:

Models E150:

Cassettes — 150
Cassette Baskets — 1
Processing Reagent Stations — 10
Paraffin Stations — 4
Cleaning Reagent Stations — 2
Fume Control Stations — 2
Reagent Bottles — 2.2 L each
Cleaning Bottles — 3.0 L each
Paraffin Tanks — 2.2 L each

Models E300:

Cassettes — 300
Cassette Baskets — 2
Processing Reagent Stations — 10
Paraffin Stations — 4
Cleaning Reagent Stations — 2
Fume Control Stations — 2
Reagent Bottles — 3.2 L each
Cleaning Bottles — 4.0 L each
Paraffin Tanks — 3.2 L each

Operating Conditions:

Temperature — 10° C to 40° C (50° F to 104° F)
Relative Humidity — 0% to 85% RH

Fluid Transfer Pressures:

Pump In — 25.0 cm Hg (nominal)
Pump Out — 0.35 kg/cm^2 (nominal)

P/V Cycle:

Pressure — 0.35 kg/cm^2
Vacuum — $\geq$ 50.0 cm Hg
90 Seconds Pressure;
30 Seconds Ambient;
90 Seconds Vacuum
30 Seconds Ambient;

Agitation:

Pump Out and Pump In Every 20 Minutes

Temperature Capabilities:

Retort Solutions — 35° C to 50° C (95° F to 122° F)
Paraffin — 45° C to 70° C (113° F to 158° F)
Paraffin Oven — 47° C to 72° C (116.6° F to 161.6° F)

Safety Standards

Tested and listed by Inchcape Testing Services (ITS).
Complies with: UL 3101-1, 1st ed.
CAN/CSA-C22.2 No. 1010.1-92

INSTALLATION

General Information

This section provides information on selecting a proper location, unpacking, and installing the Tissue-Tek® VIP™ instrument. A Tissue-Tek instrument representative or a certified Biomedical Equipment Technician should perform the installation. The instrument must be installed correctly to ensure proper operation and service. Read this Operating Manual carefully before attempting to operate the instrument. Follow all instructions carefully.

The VIP processor is a precision instrument and must be handled accordingly. Rough handling or dropping of the instrument will disturb or damage internal components. Always handle the instrument with care.

Environmental Factors

Environmental factors influence the selection of a proper location for the VIP instrument. As with all sensitive electronic instruments, prolonged exposure to excessive humidity and temperature should be avoided. Temperature and humidity should be held relatively constant to obtain the highest degree of operating stability. The ambient temperature range for operating the instrument is 10° C to 40° C (50° F to 104° F). The ambient operating humidity range is 0% to 85% relative humidity.

Locate the instrument in a *well-ventilated* area, avoiding exposure to corrosive vapors or temperature extremes. Avoid proximity to direct sunlight, open windows, sinks, ovens, open flames, hot plates, radiators, and dry ice baths. It should also be located away from any instrument that consumes a high voltage or large current, including large refrigerators and ovens. If installing a floor model, the floor must be solid and level; for bench models, the bench must have a firm level surface capable of supporting at least 181 kg (400 lbs.) of weight.

Be sure the instrument will be located near a power source that meets the electrical requirements (voltage and amperage) specified on the rating label located on the rear of the instrument. The power receptacle must be grounded and should be a clean, noise-free, dedicated line.

Unpacking

1. Unpack the instrument by removing the large nails along the bottom of the shipper, then lifting the shipper off. ***Carefully*** lift the instrument off the wooden base.

   CAUTION: The instrument is very heavy and large; therefore, it is strongly recommended that it be lifted and transported by *at least* two people.

2. **Floor Model:**
   After the instrument is unpacked, roll it to the firm, level surface in the designated work area. Once in its final position and all installation steps are complete, lock the front casters to prevent instrument movement.

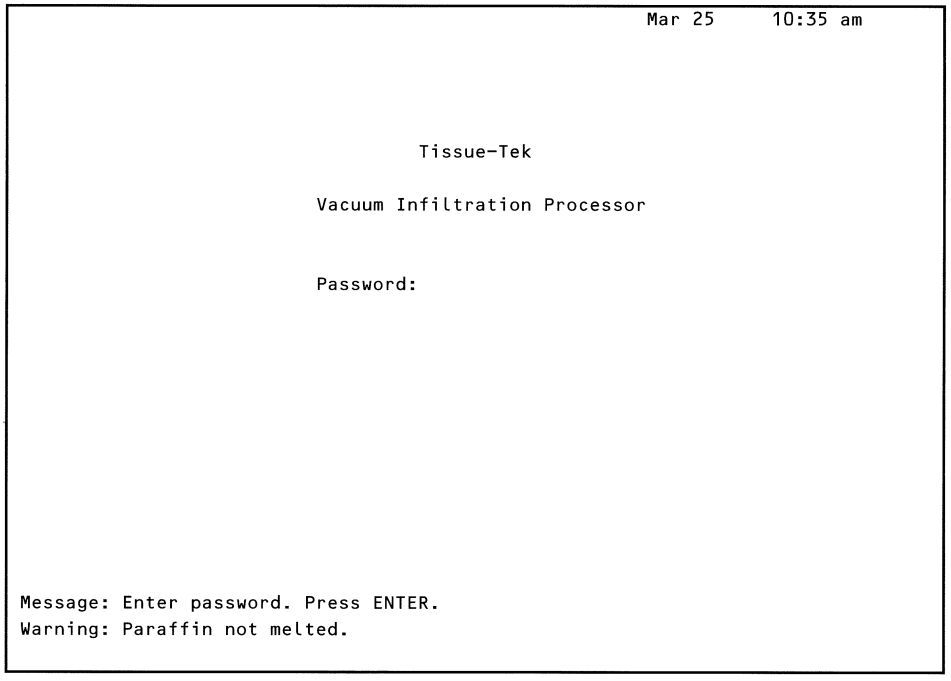
   Bench Model:
   After the instrument is unpacked, ***carefully*** lift it into place on the firm, level surface in the designated work area. Be sure that each leveling foot fully contacts the benchtop and that each foot has been properly adjusted so the instrument is level.

3. Confirm that all accessories have been included with the instrument:
   - Operating Manual — 1
     (including Warranty Registration—U.S. customers only)
   - Reagent Bottles — 13
   - Paraffin Tanks — 4
   - Activated Carbon Cartridges — 2
   - Cassette Basket (Models E150 — 1; Models E300 — 2)
   - Spill Trays — 2
   - Scraper — 1

   If any of these items are missing, contact your Customer Support Representative (refer to Section 9).

   If there is visible shipping damage to any item, immediately file a complaint with the carrier. Then, notify your nearest Tissue-Tek instrument distributor, or call Sakura directly.
4. Plug the instrument power cord into a dedicated, grounded electrical power source of the proper voltage and amperage.
5. Move the power switch, located on the rear of the instrument behind the control panel, to the ON position. A small green light on the control panel will illuminate.

   NOTE: The instrument will not begin automatic operation until approximately 24 hours after the power is turned *on*. This ensures that the oven temperature has reached and stabilized at its set temperature. (Refer to Section 3, "Check Paraffin" to override this delay.)
6. The screen on the control panel should be displaying the Initial Screen (shown below), with the cursor requesting entry of the Password. Press "0" "0" "0" "0" "ENTER" on the keypad; the display will change to the Main Menu, which includes a display of the temperature of the paraffin oven (located in the lower right corner of the display).



7. Connect the external alarm, if desired, according to the directions found in the Tissue-Tek VIP Service Manual.
8. Install the two spill trays, one in the rails directly under the oven and the other in the rails under the reagent cabinet (Figure 2-1).

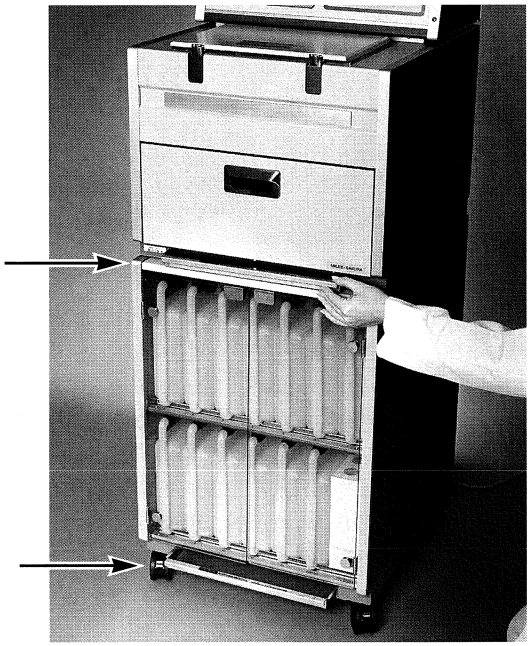


Figure 2-1

9. Locate the serial number label found on the left side of the instrument, immediately below the paraffin oven (Figure 2-2); a duplicate label is also found on the rear of the instrument. Write the installation date and instrument serial number on the Warranty Registration Card found in the front of this manual. Also write the installation date and serial number in the "Preservice Checklist" in Section 9. Completely fill out the Warranty Registration Card and mail.

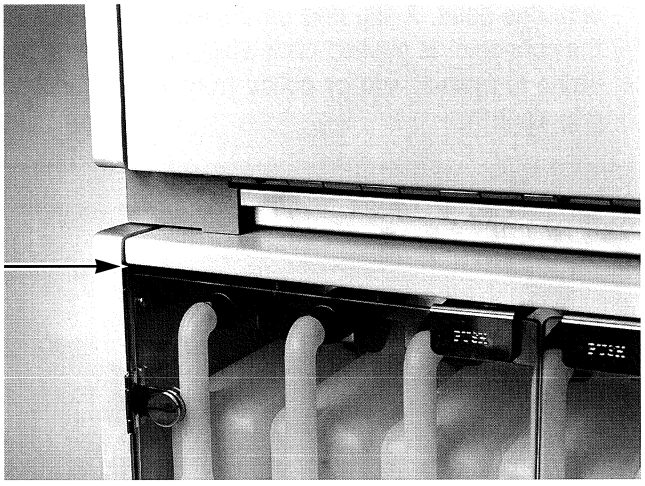


Figure 2-2

Instrument Setup

1. Open the glass doors on the front of the reagent cabinet by pressing the metal plates on the upper inside corner of each door. This disengages the lock and allows the doors to swing open.
2. The reagent bottles are connected into the instrument through "quick-release" connectors that enable the bottles to be securely connected or removed with one hand. A slip ring on the instrument side of the connector is pushed back while the nozzle on the bottle is pushed into or pulled from the connection (Figure 2-3).

Figure 2-3

Remove each bottle from the reagent cabinet by pushing against the ring on the quick-release connector, then pulling the bottle toward you (Figure 2-4).

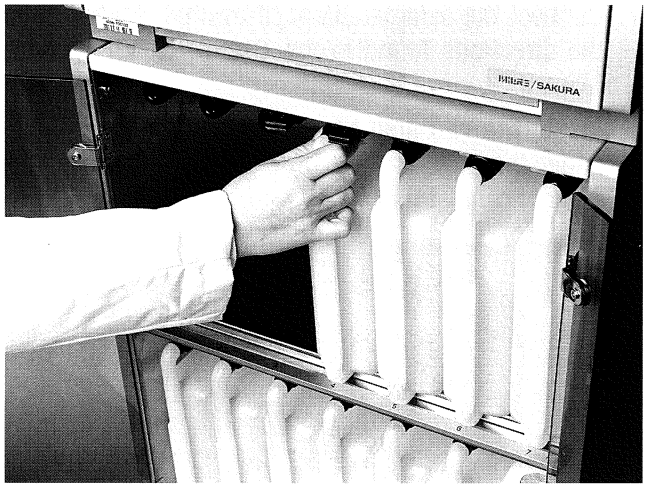


Figure 2-4

3. Examine one of the bottles for the line of graduation marks and letters molded into each side. The markings on the right side of the bottle are specific for the E300 models; those on the left side are for the E150 models (Figure 2-5). The "P" designates "Processing" volumes (2.2 L for the E150 models; 3.2 L for the E300 models); the "C" designates "Cleaning" volumes (3.0 L for the E150 models; 4.0 L for the E300 models).

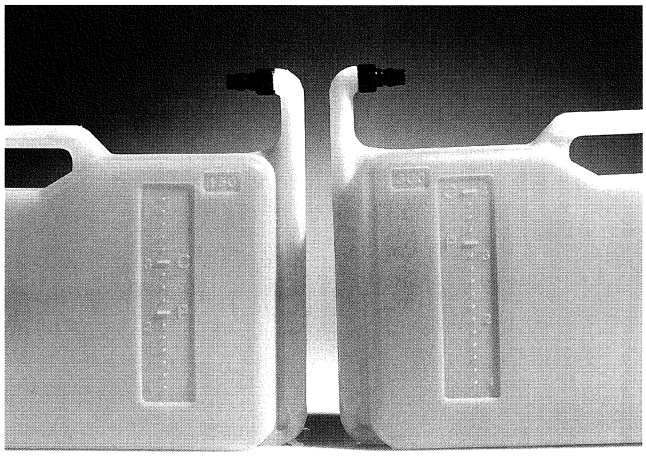


Figure 2-5

4. Label each bottle with the appropriate reagent and concentration that will be used at each station. (Later, these will be programmed into the software during the customization of the instrument, as instructed in Section 3.) Remove the cap at the opposite end of the bottle from the nozzle and fill each bottle to the proper graduation mark (to the appropriate "P" marking for Stations 1-10 and to the appropriate "C" marking for Stations 15-16) with the desired reagent. Be sure the bottle is placed on a level surface for filling. Replace the cap, ensuring that the long point on the cap aligns with the arrow on the bottle (Figure 2-6).

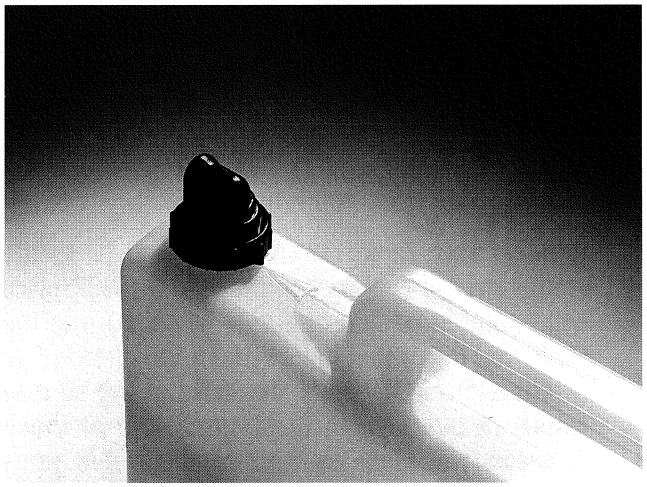


Figure 2-6

5. Finally, fill the bottle for the station labeled WATER (part of the fume control system). **This bottle is the exception for fill quantity;** fill it with water *to the "P" marking on the **left** side (2.2 L), regardless of which model (E150 or E300) you are using.* Then replace the cap in the same way as for the other bottles.
6. Replace each bottle into its proper station as follows:
   a. Carefully slide the bottle into the proper station, cap end first, until the nozzle begins to slide into the quick-release connector (Figure 2-7).

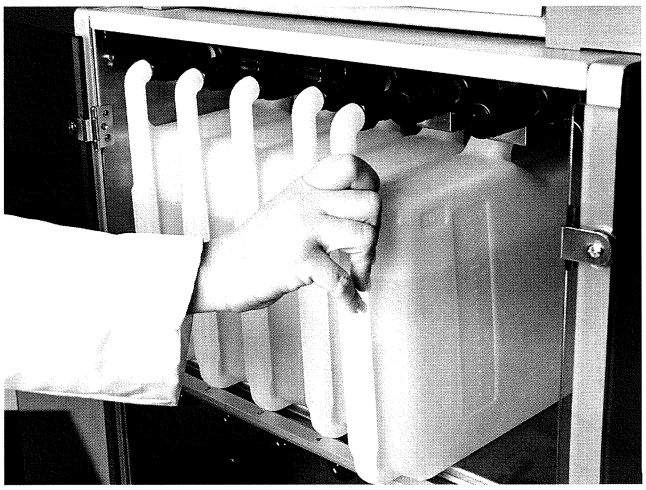


Figure 2-7

   b. Press back on the slide ring; at the same time, push the bottle in until the nozzle is fully seated in the connector (Figure 2-8), and release the slide ring.

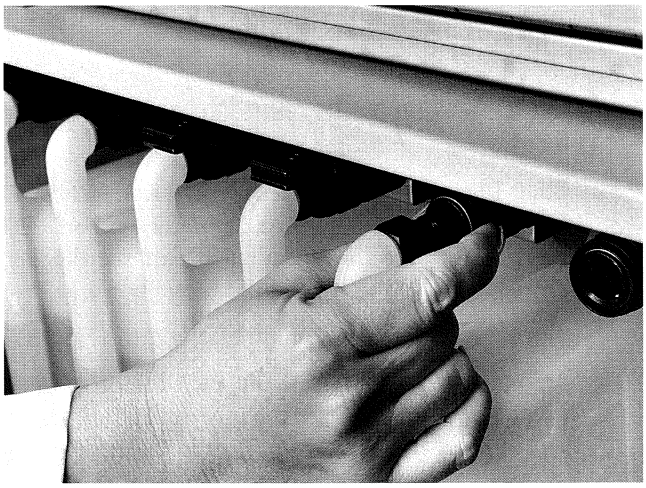


Figure 2-8

   c. *Ensure that the bottle is securely connected by gently pulling it out. It should not budge. If there is any slippage, repeat the connection.*

7. Remove the cartridge of activated carbon from its plastic bag. With the vent holes to the top and the connecting hole towards the instrument, slide the cartridge into the station labeled Activated Carbon (Figure 2-9). When a slight resistance is felt, push firmly on the cartridge to ensure a proper connection.

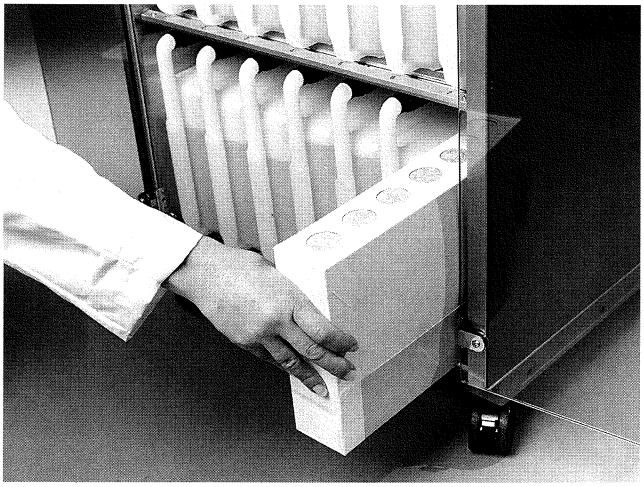


Figure 2-9

8. Adjust the tension on the retort latches, if necessary, as follows:
   a. Slide the retort bar to the left to allow access to the retort latches. "`Retort Lid unlocked`" will be displayed on the Warning line of the display. (Touch "OFF/CLEAR" key to silence the alarm.)
   b. Flip each latch down to unhook the retort lid.
   c. Adjust each latch individually, with the other latch open. For each latch, lift the inner grip of the latch so it hooks over the ledge on the lid, then press up on the other part of the latch so it locks into place. Both latches should close with the same amount of pressure; if they do not, adjust the tension by turning the inner grip clockwise to tighten or counterclockwise to loosen. The latches should close snugly but without the need for excessive force.

      NOTE: If the latches are very loose, the retort lid will not be properly sealed and processing may be stopped.
   d. Slide the retort bar to the right to cover the latches. "`Retort Lid unlocked`" should disappear from the display.
9. Monitor the temperature of the oven; the default temperature is 62° C (143.6° F). If this temperature is not acceptable for the paraffin being used, set the appropriate temperature, as described in Section 3, "Edit Programs." If it is acceptable, the paraffin tanks can be filled with previously-melted paraffin when the oven has reached its appropriate temperature.

   Remove each tank from the oven and fill to the embossed line with molten paraffin. DO NOT place solid paraffin (including flakes or chips) into the tank, as this may cause the oven heater to overheat. DO NOT overfill the tanks; doing so may cause an overflow in the retort. Both of these problems will cause an error condition that stops processing.

   WARNING: Use extreme care when filling the paraffin tanks. Molten paraffin is very hot and can cause burns.

NOTE: Although the reagent bottles are all the same, the paraffin tanks differ between Models E150 and E300. If you have both models, DO NOT interchange the tanks. See Figure 2-10 for the differences.

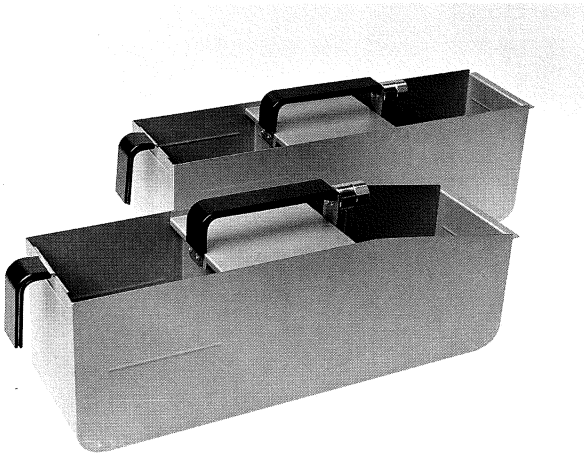


Figure 2-10

10. **CAREFULLY** replace each paraffin tank into the oven. When a slight resistance is felt, gently and carefully press in on the handle until the line connector on the tank is securely seated inside the connector in the instrument. Close the oven door.

Precautions

There are several precautions that must be observed before operating the instrument:

1. Check the fill level of each reagent bottle to ensure the specified volumes. Overfilling the bottles may cause an overflow in the retort, resulting in an error condition and halted processing.
2. Check the screw caps of each bottle to ensure tightness. Check each connection to ensure that it is properly seated.
3. Check the paraffin containers for proper fill level. Use only paraffin with the same melting point, not to exceed 70° C (158° F). Use molten paraffin only, and DO NOT overfill the paraffin containers. Ensure that all tanks are properly seated in their connections.
4. NEVER open the retort lid if "Vacuum" or "Pressure" is being displayed for the retort condition. Open the lid only when "Ambient" is being displayed.

   NOTE: The pressure and vacuum are released automatically through a safety relief valve when the retort bar is moved to the left. Wait for "Ambient" to be displayed before opening the lid.
5. Do not obstruct the ventilation louvers in the top of the control panel. This area must be kept clear to ensure proper ventilation. Also, do not place any containers of liquid on the top of the control panel; spillage could cause extensive damage to the instrument.

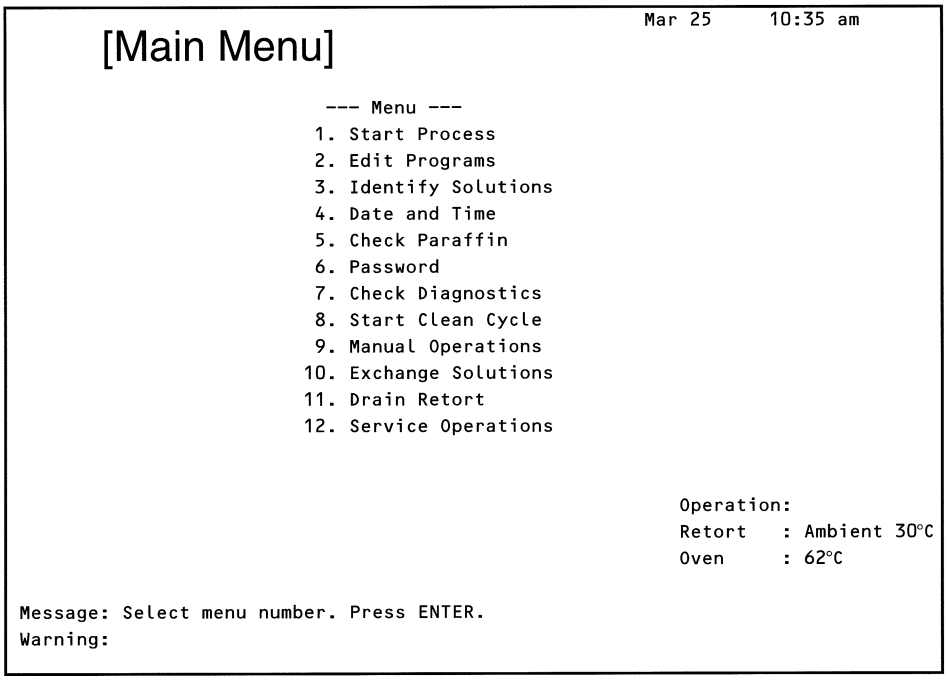
EXPLANATION OF SOFTWARE/CUSTOMIZATION

General Information

The Tissue-Tek® VIP™ Vacuum Infiltration Processor contains software that can be customized for the work routine and procedures used in the individual laboratory. This section explains the various operating modes and provides information on customizing the software. Many of the procedures in this section will be used upon initial installation and only rarely thereafter. However, detailed information on the progression of screen displays and prompts is given here; therefore, this section should be read thoroughly before beginning routine operation. Once you are familiar with this information, Section 4, OPERATING INSTRUCTIONS, can be used as a quick reference for routine operation.

When the VIP instrument is first turned *on*, the initial screen (shown in Section 2) is displayed, which requires entry of a "password" (entry code) to progress further in the displays. (This screen will also be displayed if the software exits from a processing program, such as after the Clean Cycle.) A default password code of "0000" is programmed into the instrument; enter this code, then press "ENTER." The screen will then display the Main Menu, shown below.

On most screen displays, the date and time are displayed in the upper right corner and the software routine or operating mode in the upper left corner. The retort and oven temperatures, the instrument operation and the retort condition, if applicable, are displayed in the lower right corner. The bottom two lines of the screen are reserved for the display of messages and warnings.



The Main Menu is the starting point for the routine operation of the instrument. Each menu item is explained below in a logical order of usage during the initial setup.

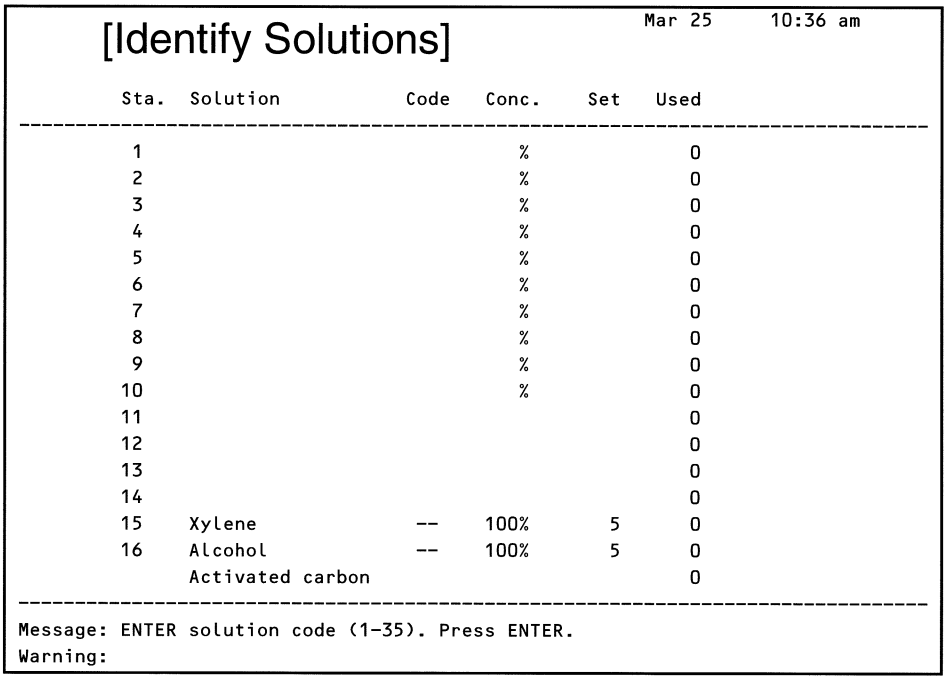
Date and Time

The date and time can be programmed by pressing "4" "ENTER" from the Main Menu. The display will show the year, date, and time that are currently programmed. Each can be changed as described below; if an entry is correct as displayed, simply press "ENTER" to continue to the next entry.

1. The cursor will initially be on the final digit of "Year," with the prompt of "`Enter year. Press ENTER.`" on the message line. If the year being displayed is incorrect, enter the correct numerical digits, then press "ENTER." (All four digits must be entered.) The cursor will move to "Date."
2. Enter the correct date (month/day), if necessary, as prompted by the message line "`Enter date (mm/dd). Press ENTER.`" A leading zero does not have to be entered for a single-digit month; however, it does have to be entered for days of less than ten. The cursor will move to "Time."
3. The message line will prompt "`Enter time. Press ENTER.`" Enter the correct time, if necessary, then press "ENTER." Again, a leading zero does not have to be entered for a single-digit hour; however, it does have to be entered for minutes of less than ten. The cursor will move to the am/pm denotation and the message line will prompt "`Press ON for AM, OFF for PM. Press ENTER.`" Press the appropriate key ("ON" or "OFF"), then press "ENTER" when the correct designation is being displayed. The display will return to the Main Menu.

Identify Solutions

One of the first steps in setting up the instrument is to identify which solutions will be used at each station. To do this, press "3" then "ENTER" from the Main Menu. The "Identify Solutions" screen will be displayed (until reagents are entered, the default screen is nearly empty):



[Identify Solutions]

Mar 25 10:36 am

Sta.	Solution	Code	Conc.	Set	Used
1			%		0
2			%		0
3			%		0
4			%		0
5			%		0
6			%		0
7			%		0
8			%		0
9			%		0
10			%		0
11					0
12					0
13					0
14					0
15	Xylene	--	100%	5	0
16	Alcohol	--	100%	5	0
	Activated carbon				0

Message: ENTER solution code (1-35). Press ENTER.
Warning:

The columns display or allow entry of, in order, the station number, solution or reagent name, code number corresponding to that particular reagent, and the concentration of the reagent. The next column, titled "Set," is used to designate how many processing cycles each reagent should be used before being replaced. The final column, "Used," shows the actual number of times that reagent was used in a completed program. With each processing cycle, the number in this column increments by one for each reagent used in the run.

When the number in the "Used" column equals the number in the "Set" column, a screen labeled "Solutions" will be displayed, along with a message alerting the user to the need to replace one or more reagents. (The "Solutions" screen is identical to the "Identify Solutions" screen except for the message line and the presence of asterisks to denote which stations need to be exchanged.) The message does not prevent the run from continuing; if bypassed, the "Used" counter will continue to increment and the message will be displayed again with the next run. The Solution Usage feature is optional and may be bypassed entirely by leaving the "Set" counts blank or pressing "CL" to clear any previously set numbers.

The reagent name in each of the first 14 stations is entered (or can be changed once an entry has been made) by entering the appropriate code number. Refer to Table 3-1 for a list of all available reagent codes. The reagents and "Set" counts listed in Stations 15 and 16 cannot be changed; only the "Used" counts can be cleared. Determine the reagents and concentrations that are to be located in each of the 14 processing stations and enter them into the instrument software, as instructed in the following steps.

1. The cursor will initially be located in the "Code" column of Station 1 and the message line will prompt "`Enter solution code (1-35). Press ENTER.`" Locate the desired reagent for Station 1 on the list in Table 3-1 and enter the code number of that reagent, using the numerical keys on the keyboard, then press "ENTER." The cursor will move to the "Conc." column.
2. The message line will prompt "`Enter solution concentration. Press ENTER.`" Enter the concentration of the reagent to be used at Station 1, then press "ENTER." The concentration can be set or cleared to any whole number from 1-100. The cursor will move to the "Set" column.
3. The prompt "`Enter set count (1-99 or CL). Press ENTER.`" will be displayed on the message line. If you do not want to use this option at all, press "CL" "ENTER," and the number of cycles will not be monitored. If you do wish to use the option, determine how many processing cycles you want to run using the reagent at this station before exchanging or replacing the reagent. Enter this number (from 1-99) and press "ENTER." The cursor will move to the "Used" column.

   NOTE: The "Set" count cannot be cleared or changed for the two cleaning reagents (Stations 15 and 16); cleaning cycles will continue to be monitored.

01	Fixative
02	Formalin
03	Formalin Substitute
04	Buffered Formalin
05	Neut. Buff. Formalin
06	Zinc Sulfate Form.
07	Alcoholic Formalin
08	Phenol-Formalin
09	VIP Fixative
10	Dehydrant
11	Alcohol
12	Ethanol
13	Methanol
14	Isopropanol
15	Butanol
16	S-29
17	VIP Dehydrant*
18	Clearing Agent
19	Clearant
20	Xylene
21	Xylene Substitute
22	Xylol
23	Toluene
24	Chloroform
25	Methanol-Chloroform
26	Benzene
27	UC-670
28	VIP Clearant*
29	Paraffin
30	VIP Paraffin
31	Wax
32	T.T. I Paraffin
33	T.T. II Paraffin
34	T.T. III Paraffin
35	Water

*This reagent is not currently available. Contact your Sakura instrument representative for further information.

Table 3-1
Reagent Codes for Model Numbers:
4890; 4892; 4894; 4896

NOTE: Countries using Model Numbers other than these four may have a different list of reagents/codes. Contact your Tissue-Tek instrument representative if this list does not agree with your software. He/She will supply you with a correct list.

4. "`Press CL to reset to '0'.`" will be displayed on the message line. If there have been no processing runs on this instrument, this column will be "0" and there is no need to clear the number. However, if runs have been performed and you plan to exchange the solution at this station, the "CL" key can be pressed to clear the "Used" count and begin again at "0." If you want to retain the displayed number, press "ENTER." The cursor will move to the "Code" column for Station 2.
5. Repeat Steps 1-4 above for each station from 2 to 10 and Steps 1, 3, and 4 for each station from 11 to 14 (the concentration of the paraffin in these stations is not set). The position of the cursor can also be moved to any desired column (to which an entry can be made) by pressing the appropriate arrow keys ($<$, $>$, $\wedge$, $\vee$). If an acceptable entry already exists in a column (or if you want to bypass an entry), the "ENTER" key can be pressed to accept (or skip) that entry.
6. When the cursor reaches the lines for Stations 15 and 16, only the "Used" column can be accessed. ONLY the reagents listed should be used at these stations, and they should always be changed after the listed number of cycles in order to ensure adequate fume control. When the cursor reaches the line for Activated Carbon, both the "Set" and "Used" columns may be accessed; to ensure proper fume control, the carbon must be changed on a regular basis, dependent upon usage (refer to Section 6). Since there is no economical way to specifically monitor the remaining absorption capacity and/or capability of the Activated Carbon, changing the Activated Carbon after every 20 runs is recommended.
7. The entries made on this screen are used as the basis for all Program screens, so examine the completed screen to ensure its correctness. The display will appear similar to the following example:

[Identify Solutions] Mar 25 10:40 am

Sta.	Solution	Code	Conc.	Set	Used
1	Buffered Formalin	4	10%	5	0
2	Buffered Formalin	4	10%	5	0
3	Ethanol	12	70%	5	0
4	Ethanol	12	80%	5	0
5	Ethanol	12	95%	5	0
6	Ethanol	12	95%	5	0
7	Ethanol	12	100%	5	0
8	Ethanol	12	100%	5	0
9	Xylene	20	100%	5	0
10	Xylene	20	100%	5	0
11	V.I.P. Paraffin	30		5	0
12	V.I.P. Paraffin	30		5	0
13	V.I.P. Paraffin	30		5	0
14	V.I.P. Paraffin	30		5	0
15	Xylene	--	100%	5	0
16	Alcohol	--	100%	5	0
	Activated carbon			20	0

Message: ENTER solution code (1-35). Press ENTER.
Warning:

8. When all reagent information has been properly entered, press the "EXIT" key to return to the Main Menu.

Password

The default password entered into the instrument is "0000." An additional password can be added if desired by the laboratory, as long as it contains a minimum of four characters (any key on the keypad can be used, with the exception of the "EXIT" key). The password can be added by pressing "6" "ENTER" from the Main Menu.

1. The message line will prompt you to "`Enter old password. Press ENTER.`" Enter the old password, pressing "ENTER" when complete.
2. The prompt will change to "`Enter new password. Press ENTER.`" Enter the new password (minimum of 4 characters).
3. Enter the new password a second time when prompted "`Reenter new password. Press ENTER.`" If the second entry matches the first entry, the display will return to the Main Menu and the new password has been stored in memory for future use. If it does not agree, four beeps will sound and the message line will briefly prompt "`They don't match. Try again.`"; the prompts will return to Step 2 above. Enter the new entry code again, then reenter it, taking care to enter the same code both times.

Edit Programs

Up to nine different processing programs can be entered into the software. The processing time and temperature can be set and the P/V cycle and Agitation options turned *on* or *off* for each station. The reagents and concentrations displayed for each station are determined by the selections made in "Identify Solutions" previously. To edit any or all of the nine programs, press "2" "ENTER" from the Main Menu.

The default conditions for Program 1 will be displayed initially; the reagents and concentrations displayed are determined by those entered through the "Identify Solutions" Routine. For example:

[Edit] Mar 25 10:42 am

--- Program 1 ---

Sta.	Solution	Conc.	Time (hr:min)	Set temp.	P/V	Agit
1	Buffered Formalin	10%	1:00	°C	off	on
2	Buffered Formalin	10%	1:00	°C	off	on
3	Ethanol	70%	1:00	°C	off	on
4	Ethanol	80%	1:00	°C	off	on
5	Ethanol	95%	1:00	°C	off	on
6	Ethanol	95%	1:00	°C	off	on
7	Ethanol	100%	1:00	°C	off	on
8	Ethanol	100%	1:00	°C	off	on
9	Xylene	100%	1:00	°C	off	on
10	Xylene	100%	1:00	°C	off	on
11	V.I.P. Paraffin		1:00	58°C	off	on
12	V.I.P. Paraffin		1:00	58°C	off	on
13	V.I.P. Paraffin		1:00	58°C	off	on
14	V.I.P. Paraffin		1:00	58°C	off	on

Endtime (days/hour:minute) 1/ 9:00 am

Message: Select program number (1-9). Press ENTER.
Warning:

1. The cursor is initially located on "Program 1" and the message line displays the prompt "`Select program number (1-9). Press ENTER.`" Enter the number of the program you wish to edit, then press "ENTER." If the program number changed, the new program will be displayed. The cursor will move to the "Time" column for Station 1.

   NOTE: The default settings for each program are identical, except for the processing time. The default timing in hours equals the program number (e.g., the "Time" column for Program 5 is "5:00" for all stations). If the same change is to be made to most or all stations, you can make the change in one step using the "Program All Stations" function (see instructions below). Also, after editing one program, you can copy that program to another, then make modifications to the copy (see "Program Copy" below).

2. The message line displays the prompt "`Enter processing time (00:00 - 99:59). Press ENTER.`" Enter the desired processing time, in hours and minutes, for Station 1, then press "ENTER." A leading zero does not have to be entered for a single-digit hour; however, it does have to be entered for minutes of less than 10. The cursor will move to the "Set temp." column.

   NOTE: If a time of "0:00" is entered for a station, that station will be skipped during the processing run; fluid will not be pumped into the retort.

3. The prompt on the message line will display "`Enter processing temperature (35-50°). Press ENTER.`" Enter the desired retort temperature for processing, from 35°-50° C (95°-122° F), then press "ENTER." The cursor will move to the "P/V" column.

   NOTE: If you do not want to have added heat at a station, press "OFF."

4. The P/V cycle (alternating pressure and vacuum) can be turned *on* or *off* for each station. The message line will prompt "`Select P/V cycle. Press ON or OFF. Press ENTER.`" Press the appropriate key, then press "ENTER" when the desired selection is displayed. The cursor will move to the "Agit" column.

5. Agitation ("Agit") can be either *on* or *off* for each station. When Agitation is *on,* the reagent will be pumped out of and back into the retort every 20 minutes; the cycle will repeat until the "Remain Time" shows 30 minutes or less. Follow the prompt "`Select agitation cycle. Press ON or OFF. Press ENTER.`" Press the appropriate key, then press "ENTER" when the desired selection is displayed. The cursor will move to the "Time" column for Station 2.

6. Repeat Steps 2-5 above for each station from 2 through 14. All prompts will be identical, with exception of the prompt for processing temperature (Step 3) for Stations 11-14. The prompt for these stations will show an allowable range of "`45-70°C`," rather than 35-50° C (95°-122° F); also the heat cannot be turned *off* for these stations. The position of the cursor can also be moved to any desired column (to which an entry can be made) by pressing the appropriate arrow keys (<, >, ∧, ∨). The "ENTER" key can be pressed to accept an existing entry and move the cursor to the next programmable field or option.

   NOTE: Check the melting temperature of the paraffin to be used before programming the processing temperature for Stations 11-14 to ensure an appropriate entry.

7. Finally, if the program will ever be used with a delayed starting time, the End time is selected. The end time is the time at which you want the processing to be completed. The instrument then determines the start time by adding the processing times entered in the program and the times required for pumping each solution in and out, then subtracting the total from the selected end time.
   a. The message line first displays "`Select number of days until completion of process. Press ENTER.`" and the cursor will be located on the day of the end time display. Count the number of times midnight will be passed from when the instrument will be started in the Delay Mode until the desired completion of the process. For this entry, each passing of midnight equals one day. Enter the correct number of days (midnights), then press "ENTER." The cursor moves to the time.
   b. The message line will change to the prompt "`Select time of day process is to be completed (hh:mm). Press ENTER.`" Enter the time at which you want the run to be completed, then press "ENTER." The cursor moves to the am/pm selection.
   c. The message line changes to "`Press ON for AM, OFF for PM. Press ENTER.`" Press the appropriate key ("ON" or "OFF") for the correct designation, then press "ENTER."

Example: If you want to program a run in which the retort is loaded on Monday, and you want processing to be completed on Tuesday at 8:00 am, you must enter "1" for the days and "8:00 am" for the time. For a run placed into the instrument on Friday to be completed on Monday at 1:30 pm, you must enter "3" for the days and "1:30 pm" for the time.

NOTE: If the processing times that were programmed earlier cannot be achieved by the end time just entered, the instrument will beep three times and the message line will momentarily display "`Check program. Processing cannot be completed as programmed.`" This message is dependent upon the *current* time. If you are editing the program for later use, simply verify that the times are all appropriate for your use and continue; the programmed end time will be accepted regardless. However, if the program is being edited *during* a processing run, or this message appears when attempting to start a new run, either the end time or one or more of the processing times must be changed in order to process in the Delay Mode.

8. The cursor will return to the beginning of the program. Review the program carefully to ensure that all information is correct. Use the arrow keys ($<$, $>$, $\wedge$, $\vee$) to move the cursor to correct any errors. When finished, either press EXIT to return to the Main Menu or press a number key to edit another program.

Program All Stations

This subfunction of the "Edit Program" Routine allows the user to quickly make the same change to all stations. It can be used for each of the four parameters that can be set in each program (time, temperature, P/V, and agitation). If only a few of the stations will be different, you can use this function to change them all, then modify those few stations as necessary.

1. To use this function, the display must be in the "Edit Programs" routine and the cursor must be located anywhere other than on the Program number. Then press "`*`" "ENTER." The cursor will be located at the bottom of the column in which it was previously located (or under "Time" if it was located at the End time selection) and "`All Stations`" will be displayed at the left side of that line.

2. Enter the desired selection and press "ENTER", in the same manner as in "Edit Programs" above. All stations will be changed to reflect that selection and the cursor will move to the next column. The < and > arrow keys will also move the cursor to the desired column.

   NOTE: The entry for "Set temp." column will affect only Stations 1-10; Stations 11-14 must be set individually through the normal "Edit Program" Routine.

3. After the "ENTER" key is pressed for the "Agit" column, the display will return to the normal "Edit Programs" screen. Or, the "EXIT" key can be pressed to return immediately. Make any changes to specific stations at this time.

Program Copy

This function, which is also a subfunction of the "Edit Programs" routine, can be used to copy one program to another. This is a convenient and quick way to set up a second program that will be very similar to another, with only a change, for example, in the end time or in the processing times.

1. To use this function, you must be in the "Edit Programs" routine, with the **NEW** program to be created on the display. This can be done either immediately upon entering the routine or immediately after the first program has been edited, as follows: If not already there, move the cursor to the Program number line, using the ∧ key. Enter the number for the program you want to ***create*** and press "ENTER." The display will change to the program screen that was entered.
2. Press "*" "1" to enter the "Copy" subroutine. The cursor will move to the bottom of the display, next to the prompt "`Copy from program 1`"; the message line will prompt "`Enter source program number (1-9). Press ENTER.`" Enter the program number that you want to copy ***from*** and press "ENTER."
3. The message line prompt will change to "`Are you sure? Press ON/YES or OFF/CL.`" Make sure the program number entered is the one you want to copy into the new screen. If it is correct, press "YES"; if not, press "CL" and the display will return to the "Edit Programs" screen, from which you must begin again at Step 2 above.
4. If "YES" is pressed, the display will change to incorporate all the parameters of the source program that was selected. You can now modify the new program to the specific parameters needed, either by selecting individual stations/parameters or by using the "Program All Stations" function ("*" "ENTER").

Check Paraffin

Although the instrument will not allow processing to begin automatically until 24 hours have elapsed after the power is initially turned *on,* the following routine will override this function. If the retort and oven temperatures have reached the specified limit and you verify to the instrument that the paraffin is molten, you will be allowed to begin processing.

1. From the Main Menu, press "5" "ENTER" for the "Check Paraffin" routine. The display will change to display the prompt:

```
Check paraffin containers.

Has paraffin melted?


Message: Press ON for YES, or EXIT
Warning: STANDBY CONDITION, Paraffin not melted.
```

2. If the paraffin in all containers is melted, press "ON/YES" and the warning line will disappear. You will be allowed to begin processing when the display returns to the Main Menu. If it is not melted, press "EXIT" and the display will return to the Main Menu with the Warning line still displayed.
   CAUTION: Do NOT attempt to begin processing unless the paraffin is completely melted. To do otherwise could cause extensive damage to the instrument.

Start Process

This menu selection is the starting point of each processing run. You cannot gain access to this routine unless the oven and retort temperatures are at the specified level, the retort is empty, the clean cycle has been performed, if necessary, and no errors exist. If any of these conditions are not met, four rapid beeps will sound and an appropriate message will be displayed on the "Warning" line at the bottom of the display.

1. To begin processing, press "1" "ENTER" from the Main Menu. The screen will display the Program number that was last used, as shown in the following example:

```
[Process]                                          Mar 25     10:50 am
                                                Experiment number 00000000
 --- Program 1 ---              Set time  Remain time
 Sta. Solution          Conc.  (hr:min)   (hr:min)    Set temp.   P/V      Agit
-------------------------------------------------------------------------------
   1  Buffered Formalin  10%     2:00       2:00        40°C       on       on
   2  Buffered Formalin  10%     2:00       2:00        40°C       on       on
   3  Ethanol            70%     0:30       0:30        40°C       on       on
   4  Ethanol            80%     0:30       0:30        40°C       on       on
   5  Ethanol            95%     0:45       0:45        40°C       on       on
   6  Ethanol            95%     0:45       0:45        40°C       on       on
   7  Ethanol           100%     0:45       0:45        40°C       on       on
   8  Ethanol           100%     0:45       0:45        40°C       on       on
   9  Xylene            100%     0:45       0:45        40°C       on       on
  10  Xylene            100%     0:45       0:45        40°C       on       on
  11  V.I.P. Paraffin            0:30       0:30        58°C       on       on
  12  V.I.P. Paraffin            0:30       0:30        58°C       on       on
  13  V.I.P. Paraffin            0:30       0:30        58°C       on       on
  14  V.I.P. Paraffin            0:30       0:30        58°C       on       on
        Endtime(days/hour:minute)    1/   8:00 am
-------------------------------------------------------------------------------
Start Mode     1 (1:Delay 2:Immediate)                Retort     : Ambient 40°C
                                                      Oven       : 60°C
Message: Select program number (1-9). Press ENTER.
Warning:
```

2. If this is the desired program, press "ENTER." If it is not, enter the desired program number and press "ENTER."
3. The cursor will move to the bottom of the screen, to the prompt "`Start Mode (1:Delay 2:Immediate).`" The message line will display "`Select start mode (1 or 2). Press ENTER.`" If you want to begin processing immediately, press "2." If you want to delay the start time of the processing (using the End time specified in the Program), press "1." After verifying the selection, press "ENTER."
4. The cursor will move to the top, right side of the screen, to the "Experiment number," with a message line of "`Enter experiment number. Press ENTER.`" If you wish to use an experiment number, enter up to 8 digits, then press "ENTER." Otherwise, simply press "ENTER" to bypass this option.
5. The message line will then prompt, "`Press START to begin or EXIT to cancel.`" If you are ready to activate the program (in either the Delay or Immediate Mode) press "START"; pressing "EXIT" will return the display to the Main Menu.
6. When "START" is pressed the first reagent of the program is pumped into the retort. The same program screen will be displayed; however, the bottom portion of the screen will also display different and additional information. For example:

```
                                Operation:Pump In
                                Retort   :Vacuum 40°C
                                Oven     :60°C
Start time          Mar 25  5:00 pm
Predicted end time  Mar 26  7:37 am
Message:
Warning:
```

   The highlighted station indicates which reagent is in current processing use.

   If the Start Mode was selected as "Immediate," [Process] will display in the upper left corner of the screen and the program will begin to time down. The Operation (Pump In, Pump Out, Move Station) and condition under which the retort is operating (Vacuum, Ambient, or Pressure) are displayed, as are the Start time and Predicted End time.

   If the "Delay" Start Mode was selected, [Delay] will display in the upper left corner of the screen until the VIP time-of-day display (upper right screen) matches the program Start time. The screen mode will then change to [Process] and the conditions applied to the program (time, temperature, pressure/vacuum, agitation) will be active.

Drain Retort

Once the processing run is complete, the retort will remain filled with the reagent from the last station for which time was entered in the program (generally, this will be paraffin). The retort must be drained and the specimen baskets removed. If paraffin has been pumped into the retort, the retort must also be cleaned (see "Start Clean Cycle" below). If any processing mode or manual operation is attempted while the retort is not empty, the instrument will emit four rapid beeps; at all times, the Warning "`Retort is not empty`" will be flashing.

1. The display returns to the Process screen when a processing run is complete, and the message "`Process finished. Press ENTER.`" is displayed. The last station at which processing was done is highlighted.
2. Press "ENTER," then follow the directions in the next prompt, "`To drain retort, Press START.`"

   NOTE: If the display is at any other screen, the warning "`Retort is not empty.`" will flash. Return to the Main Menu if necessary by pressing "EXIT," then press "1" "1" "ENTER" for the "Drain Retort" option. The prompt will state "`To begin operation, Press START.`"

3. The Pump-Out cycle will begin immediately upon pressing "START." After it is complete, the "Clean" screen (for the Clean Cycle) will be displayed. Remove the specimen baskets from the retort before starting the Clean Cycle.

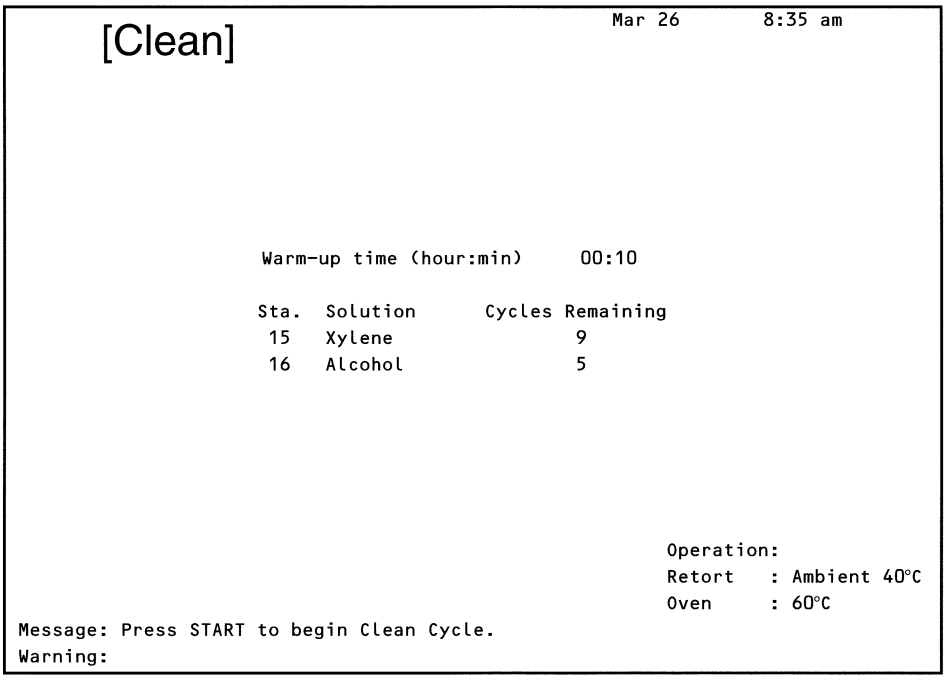
Start Clean Cycle

Any time reagents from Stations 11-14 (paraffin) have been pumped into the retort (or a pump-in was attempted), the retort must be cleaned before any additional processing cycles can be started. However, the instrument will not require that a cleaning cycle be performed unless a station higher than Station 10 has been used.

NOTE: If paraffin was not pumped into the retort, the retort should be wiped out with a dry cloth regardless of whether or not a Clean Cycle is performed. This will ensure that remnants of the final reagent in the retort do not contaminate other reagents.

1. From the Main Menu, press "8" "ENTER." The display will show the following:

   NOTE: If a processing run was just completed and the retort drained, this screen will already be displayed.



2. If you are ready to begin the Clean Cycle, press "START." If cleaning is not necessary, or if you wish to delay performing the Clean Cycle, press "EXIT." You will be prompted to enter the password to prevent unauthorized personnel from starting a run or changing the processing parameters. After entry of the password, the display will return to the Main Menu.
3. Upon pressing START, the following will occur:
   a. A warming time of 10:00 minutes begins, during which time the retort is heated to 65° C (149° F) in order to melt the paraffin remaining in the retort. The display constantly updates the amount of time remaining.
   b. After the warming time has elapsed, the instrument pumps out the melted paraffin from the retort into the last paraffin station for which processing time was programmed (generally Station 14).
      NOTE: If paraffin had not been pumped into the retort, the instrument performs the pump-out cycle into the cleaning xylene (Station 15).
   c. The instrument then begins the first of nine pump-in/pump-out cycles with xylene (Station 15). The number of cycles remaining is displayed on the screen.
   d. After all xylene cycles have been completed, the first of five alcohol cycles begins (Station 16). Again, the number of cycles remaining is displayed.
   e. After the final alcohol cycle has been completed, the display returns to one of two screens:
      i. to the "Solutions" screen if there are any solutions that need to be exchanged (see "Exchange Solutions" below), or
      ii. to the Main Menu if no solutions require exchanging.

Exchange Solutions

Reagents gradually become diluted and deteriorate after repeated uses. To keep a consistency of reagent purity some users may wish to "rotate" their reagents . . . discard the first reagent of a particular group or set and then move the following reagents nearer to the beginning of the cycle or to be the first used reagent of that particular set. The reagent container which held the discarded reagent should be cleaned and/or rinsed with clean reagent. Then a full bottle of "new" reagent is placed at the end of that particular set of reagents (or that reagent grouping). The freshest reagent (or purist) of a group will always be the final reagent to complete that particular chemical exposure to or infiltration of the specimens.

This process can be done manually by physically changing the bottle locations, or it can be done automatically by the instrument through the "Exchange Solutions" Mode. Two different groups of reagents, from Station 1 to Station 10, can be programmed for automatic exchange. Exchanging reagents between two stations requires three to five minutes; exchanging all ten stations requires about 40 minutes.

If a "Set" count was entered through the "Identify Solutions" screen, the software will automatically notify you when it is time to exchange solutions. When the number of runs performed (used) equals the number of runs selected as "set," the "Solutions" screen will be displayed, with each station requiring exchange denoted by asterisks (*) at both the beginning and the end of the display line. (The "Solutions" screen is identical to the "Identify Solutions" screen except for the message line and the asterisks.) This notification includes Stations 11-16 and the active carbon, even though automatic exchange cannot be done for these stations.

1. If the display is showing the "Solutions" screen, press "EXIT" to return to the Main Menu, then press "1" "0" "ENTER" to enter the Exchange Mode. The display will show the first ten stations, with those requiring exchange showing an asterisk at the end of the line. If exchange groups have been previously entered, those designations will be displayed. For example:

[Exchange Solutions] Mar 26 12:35 am

	Sta.	Solution	Code	Conc.	Set	Used
	1	Buffered Formalin	4	10%	5	0
	2	Buffered Formalin	4	10%	5	0
First1	3	Ethanol	12	70%	5	5*
	4	Ethanol	12	80%	5	5*
	5	Ethanol	12	95%	5	5*
	6	Ethanol	12	95%	5	5*
	7	Ethanol	12	100%	5	5*
Last1	8	Ethanol	12	100%	5	5*
First2	9	Xylene	20	100%	5	5*
Last2	10	Xylene	20	100%	5	5*

Station 16

Operation:
Retort : Ambient 35°C
Oven : 60° C

Message: If exchange sequence is correct, press START, otherwise press CL.
Warning:

2. If the Exchange Mode has not been previously used, there will be no exchange group designations displayed to the left of the station number. They must be entered the first time, as explained in Steps 3-5 below. Once the groups have been entered, they will be displayed each time the Exchange Solution Mode is entered. If they are correct for the desired exchange, simply press "START." The explanation of the operation is explained beginning in Step 6 below. If the displayed groups are not correct for the desired exchange, they can be edited, as explained in Steps 3-5 below.
3. If the exchange groups are not displayed or are incorrect for the desired exchange, press "CL." Any previous entries will be erased, and the message line will state "`Highlight first station to be exchanged. Press ENTER.`" Use the ∧ and ∨ arrow keys to move the highlighted bar to the first (lowest numbered) station in the group to be exchanged. Press "ENTER" when the appropriate station is highlighted. The station will be labeled as "First1."
4. The message line will prompt "`Highlight last station to be exchanged. Press ENTER.`" Use the ∨ key to move the highlighted bar to the last (highest numbered) station in the group to be exchanged and press "ENTER." The station will be labeled as "Last1." Together, the two stations represent the first exchange group.

   NOTE: The reagent codes within a group must be the same or four beeps will sound and the message "`Different solution in same group`" will be briefly displayed. All previous entries will be deleted and you must begin again with Step 3.
5. The message line will ask "`Exchange a second group? Press ON/YES or OFF/CL.`" If you want to designate a second exchange group (generally, for another reagent), press "ON/YES" and repeat Steps 3 and 4 above. The stations will be labeled as "First2" and "Last2." If you only want to designate one group, press "OFF/CL" and the prompts will continue as explained below.
6. If only one exchange group is designated, the message line will prompt "`Empty container A. Press START.`" (A = the station designated as First1.) If two groups have been designated, the line prompts "`Empty containers A and B. Press START.`" (A = the station designated as First1; B = the station designated as First2.) Empty the appropriate container(s), replace into the cabinet, and press "START." The Exchange Cycle will begin immediately, performing the steps shown in the following example:
   a. Assume that Group 1 designates Stations 3-8 (First1 = Station 3; Last1 = Station 8) and Group 2 is Stations 9-10 (First2 = Station 9; Last2 = Station 10). Beginning with Group 1, a pump-in will be attempted from Station 3 (First1) to ensure the bottle is empty. If it is not, the message line will state "`Container not empty.`" Press the "START" key again after emptying the bottle.
   b. Exchange will then begin. The reagent in the bottle at Station 4 will be pumped into the retort, then pumped out of the retort into the bottle at Station 3. Station 5 will then be pumped into Station 4 (via the retort), and so forth. Arrows will be displayed next to the two stations being exchanged, ← for the station from which the reagent is being pumped, and → for the station to which it is going.

c. When the final station has been pumped (Station 8 into Station 7), the process will repeat (steps a and b above) for Group 2.

d. When exchange of the final group is complete, the instrument will perform a pump-in and pump-out from Station 16 (the cleaning alcohol). The message line will then prompt "`Exchange complete. Fill containers X and Y.`" (X = the station designated as Last1; Y = the station designated as Last2.) (If only one group was exchanged, the message line reads "`Exchange complete. Fill container X.`")

e. The "Used" column automatically resets to "0" for those stations that were exchanged.

7. Fill the appropriate container(s) with fresh reagent of the correct concentration. Then press "ENTER"; the display will require you to enter the password, then will return to the Main Menu.

Manual Operations

The Manual Mode is not used during normal operation of the instrument; however, manual operation may sometimes be necessary. Three functions can be achieved through the Manual Mode:

- Pump-Out Empties a reagent from the retort;
- Pump-In Brings a reagent into the retort;
- Change Station Moves the rotary valve to the next station.

1. To enter the Manual Mode, press "9" "ENTER" from the Main Menu. The display will show the following menu:

```
1. Pump Out
2. Pump In
3. Change Station
```

   The message line will prompt "`Select menu number. Press ON.`"

2. Select the desired function by pressing the appropriate numerical key, then press "ON" to begin the operation. (Pressing "START" or "ENTER" also starts the operation.) The function begins immediately, pumping the reagent into the retort from the station displayed, pumping the reagent out of the retort into the station displayed, or moving the rotary valve to the next station.

3. When the operation is complete, you can select another operation by pressing the appropriate key and "ENTER," or repeat the "Change Station" operation by merely pressing "ENTER."

   NOTE: If a pump-in was performed (that is, fluid was pumped into the retort), you must perform a pump-out before you will be allowed to change stations or to exit the Manual Mode.

4. Press "EXIT" to return to the Main Menu when you no longer require manual operation of the instrument.

Check Diagnostics

If an error occurs during a processing run, information about each error is displayed through the "Check Diagnostics" function. Each occurrence of an error is stored in sequential order for later display; the Warning line on the display will flash the message "`Errors. xx, xx,`" with each error number listed.

When errors occur during a processing run, the run will be affected in one of three ways, depending upon the type of error:

- The instrument corrects the error and the processing run continues; however, the occurrence may have delayed the scheduled end time.
- The processing run is stopped; the error can be corrected by the operator and the run continued by following the prompts given on the message line of the display.
- The processing run is stopped and the error cannot be corrected without ending the run.

Errors that stop the run will cause an alarm to sound for 10 seconds every minute until the instrument is attended (and will set off the external alarm in five minutes if one is connected). Pressing "OFF" will silence the internal alarm; moving the retort bar to the left, then to the right, will silence the external alarm.

If a critical error has occurred that causes the run to be aborted, the display will flash the message "`Operation interrupted. Press any key.`"; the station line at which processing stopped will be highlighted, and the error numbers will be displayed on the warning line. The "Check Diagnostics" function can be utilized to help determine the cause of the error. Press any key; the processing run will end and the display will return to the Main Menu.

1. Enter the Diagnostics Function by pressing "7" "ENTER" from the Main Menu. (It can also be entered during a processing run through the Stop Menu. See "Interrupting the Run" in Section 4.) The "Check Diagnostics" screen will be displayed, with the following information for each error:

Display	**Explanation**
Error	Error number
Sta.	Station number at which error occurred
Retort temp.	Temperature of the retort
Oven temp.	Temperature of the paraffin oven
Rotary Valve temp.	Temperature of the rotary valve
Time	Time at which the error occurred

2. Refer to Section 7, TROUBLESHOOTING, for a complete explanation of each error code that is displayed.
3. Record or print the screen information, then, if desired, press "CL" to clear the error messages on the "Check Diagnostics" screen. Press "EXIT" to return to the previous screen. The error codes will also clear automatically when the next processing run is started.
4. If the processing run was ended prematurely, return to the Main Menu, then enter the "Edit Programs" Routine by pressing "2" "ENTER." Edit the program that was being used, entering a processing time of "0:00" for all stations for which processing was complete. Start the run over using the Immediate Mode. After processing is complete, reedit the program to the original processing times.

Service Operations

The final menu item on the Main Menu is "Service Operations." This routine requires entry of a special password in order to access the functions. It is designed for use by Tissue-Tek Instrument Service Engineers and other authorized personnel only.

Help Menu

There are several special functions and routines that are accessed through the use of the "*" key plus a second key. These are listed by pressing "*" "<":

Keys	Function	Description
* V	Print data	Prints data to the optional printer.
* <	Display help menu	Displays the Help Menu.
* >	Monitor	Displays the Monitor function (see "Monitor Display" below).
* ENTER	Program all stations	Enters the specified parameter into all stations during the Edit Programs Routine.
* 1	Program copy	Copies an existing program to another program number for quick editing during the Edit Programs Routine.

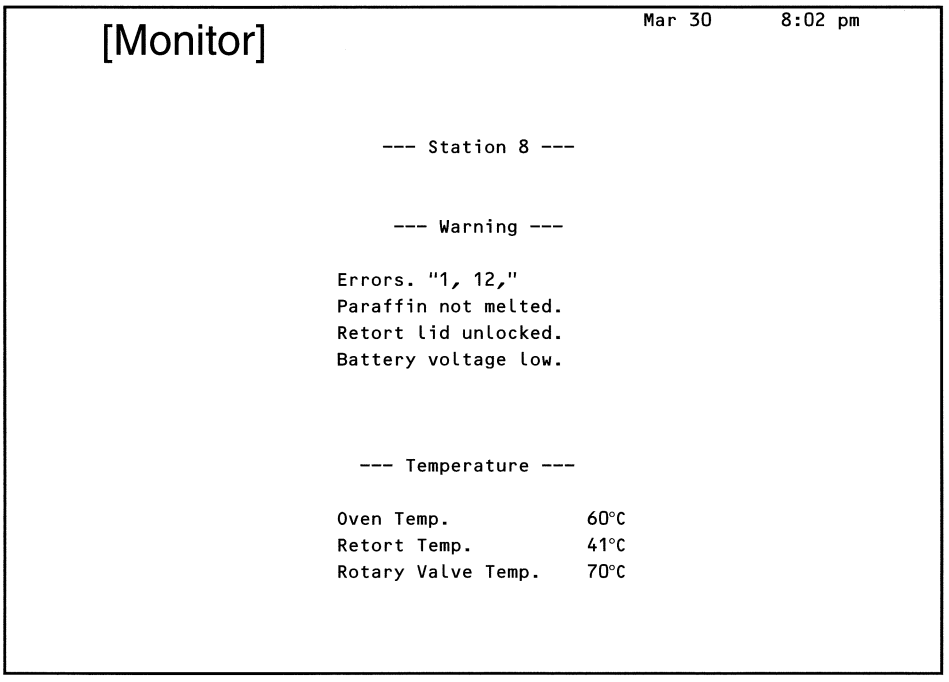
The Help Menu will be displayed for 30 seconds before the screen returns to the previous display. Pressing "EXIT" will return the display immediately.

Monitor Display

The Monitor Display requires pressing "*" ">" in order to gain entry. You can access this display from the following modes and routines:

Main Menu	Drain Retort
Edit	Check Diagnostics
Manual	Process (in action)
Clean (in action)	Exchange Solution (in action)

The screen will display the station at which the rotary valve is located, any warnings that have been displayed since the beginning of the processing run, and the temperatures of the oven, retort, and rotary valve, as shown in the example below. The display will revert back to the previous screen after 30 seconds.



OPERATING INSTRUCTIONS

Routine Operation

Once the instrument software has been customized for your laboratory needs, the Tissue-Tek® VIP™ Vacuum Infiltration Processor is ready for use in routine processing. The instrument is designed to be left *on* at all times, as this maintains the oven temperature to prevent solidification of the paraffin. This section provides the steps to follow for each processing run.

1. Ensure that the retort is drained and the Clean Cycle has been run if paraffin was pumped into the retort during the previous run (see Steps 7 and 8 below). These steps must be performed before the instrument will allow a new processing run to be performed.
2. Prepare the specimens for processing as follows:
   a. Properly fill and seal the Tissue-Tek® Uni-Cassettes® or Process/Embedding Cassettes, ensuring that there is a sufficient ratio between the size of the specimen and the volume of the cassette.
   b. Place the divider into the sample basket, then load the cassettes with the slanted end up (Figure 4-1). Each partition holds up to ten cassettes. Close and latch the basket lid. Fill the second basket if applicable.

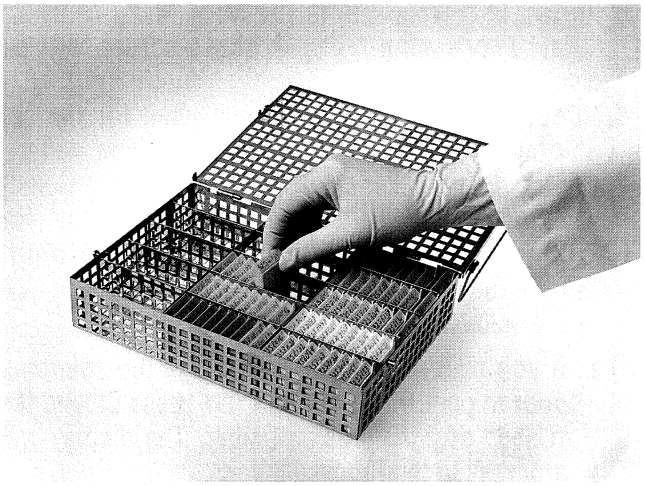


Figure 4-1

   c. Open the retort by sliding the retort bar to the left and unhooking the retort latches. Place the basket(s) into the retort, rotating the handles so they lay flat against the basket lid (Figure 4-2). Close and latch the retort lid, then slide the retort bar to the right. Make sure the message "`Retort Lid unlocked`" has disappeared from the display.

   NOTE: Before loading the retort, check that it is empty of any liquid and that the inside is clean.

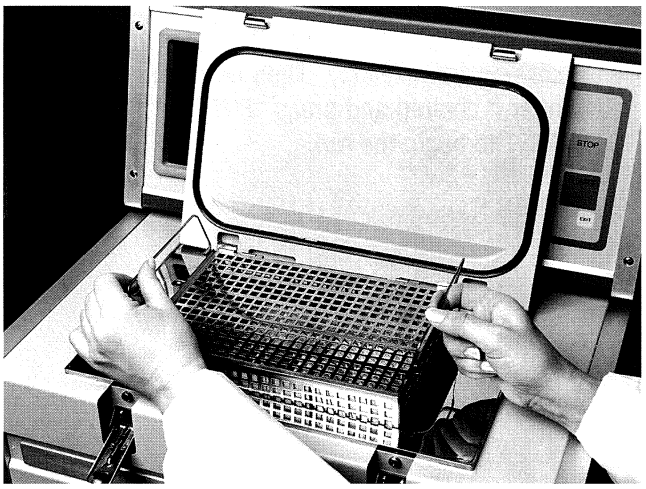


Figure 4-2

3. Press "1" "ENTER" from the Main Menu to select "Start Processing." The Process screen will be displayed.
   NOTE: If any solution has been used for the set number of runs, the "Solutions" screen will be displayed, with asterisks (*) placed to either side of the line for those stations that are due for exchanging. The message line will prompt "Exchange marked solution. Press CL to reset counts."
   a. If you plan to manually exchange the solutions prior to performing another run, press CL and the "Used" designations will clear. The display will progress to the Process Screen.
   b. To exchange the solutions using the Exchange Mode, press "EXIT" "EXIT" to return to the Main Menu, then press "1" "0" "ENTER" for the Exchange Mode. **Be sure to remove the specimen baskets before starting the exchange.**
   c. If you wish to begin processing without exchanging the solutions, press "EXIT"; the used count will continue incrementing. Again, the Process screen will be displayed; however, the used count will not be cleared.
4. Select the number of the program you want to use, then press "ENTER." Press "1" if you want to delay the start time of the run, or "2" if you want to begin processing immediately. Then enter the Experiment Number if desired and press "ENTER." Finally, press "START" to begin the run.
5. a. If "Immediate" was selected, the screen will show "Process" at the top; the current time will be displayed as the "Start Time" and the predicted End time will be calculated and displayed on the next line. The rotary valve will return to Station 1, if necessary, and processing will begin immediately, starting with Station 1 (or the first station for which time is programmed).
   b. If "Delay" was selected, the screen will show "Delay" at the top, and the Start Time will be highlighted. The rotary valve will return to Station 1, if necessary, and the reagent in Station 1 (or the first station for which time is programmed) will be pumped into the retort to prevent deterioration of the samples. When the Start Time is reached, processing will begin; the screen display will change to "Process."
6. When processing is complete, the message line will display "Process finished. Press ENTER." The instrument will beep intermittently for approximately 30 seconds. The highlighted bar will remain on the final station for which time was entered in the program.
7. After pressing "ENTER," the message line will display "To drain retort, Press START." A pump-out cycle will begin immediately after pressing "START."

8. The display will then show the screen for the Clean Cycle, with the prompt "`Press START to begin clean cycle.`" Open the retort and remove the basket(s) of specimens. Use the plastic scraper to scrape any solidified paraffin from the sides and lid of the retort. If desired, replace the *empty* baskets into the retort for cleaning. Close the retort, then press "START" to begin the Clean Cycle. When the Clean Cycle is complete, the screen will request entry of the password to prevent unauthorized access to the instrument; after the password is entered, the Main Menu will be displayed.

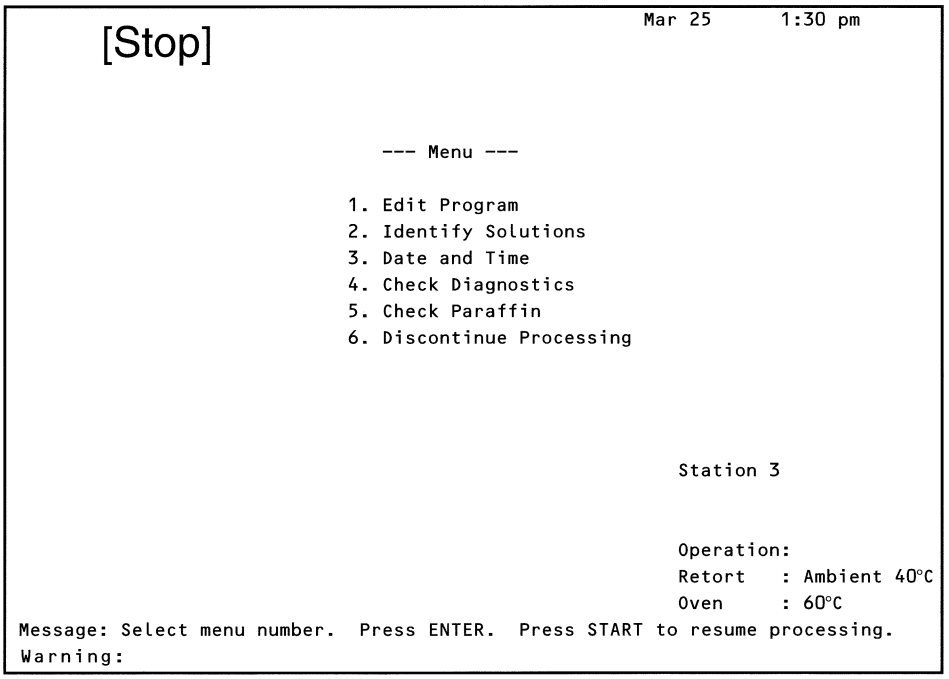
   NOTE 1: Metal items, such as base molds and forceps, may also be placed in the retort for cleaning. However, if additional items are cleaned in this manner, the cleaning reagents (Stations 15 and 16) may have to be replaced more often.

   NOTE 2: If reagent from any of Stations 11-14 was pumped into the retort, the Clean Cycle MUST be performed before another processing run can be started. However, if paraffin has not been used, and you do not wish to perform the Clean Cycle, press "EXIT." The software will then require you to enter the password in order to bypass the Clean Cycle. After entering the password and pressing "ENTER," the Main Menu will be displayed. Wipe out the retort with a dry cloth before performing another processing run if the Clean Cycle was not performed; this will help prevent contamination of the reagents from remnants of the final reagent pumped out of the retort.

Interrupting the Run

A processing run can be interrupted, for example, in order to edit the program or discontinue the run. If the run is not discontinued, processing can be started again after the editing function by pressing "START."

1. To interrupt a run that is either processing or in the Delay mode, press "STOP." The message line will prompt "`Enter password or processing will resume in 30 seconds.`" You must enter the password within 30 seconds or processing will begin again. Enter the password and press "ENTER."
2. The display will change to the Stop screen:



3. Press the appropriate numerical key for the desired function, then press "ENTER." Each function is explained below. The screens displayed and the operation of each function are identical to those seen in the routine when it is selected from the Main Menu.

Edit Program

If "Edit Program" is selected, the display will change to the Edit screen for the program being used for the processing run. The time, retort temperature, P/V, or agitation selections can be changed for any station not yet completed; in addition, the end time can be changed. When all necessary changes have been made, press "EXIT" to return to the Stop screen.

NOTE: The changes made affect *only* the current processing run; they do not affect the parameters stored in memory for the program.

Identify Solutions

The solution names, concentration, and "Set" designations can be changed if "Identify Solutions" is selected. When all necessary changes have been made, press "EXIT" to return to the Stop screen.

NOTE: The changes made remain in effect as part of the parameters stored in memory for *this and all* programs.

Date and Time

The current date and time can be corrected during the run, if necessary, by selecting "Date and Time" from the Stop Menu. If the new date or time changes the end time or start time of the processing run, this will be reflected in the times displayed on the Process or Delay screen. The display will automatically return to the Stop menu if the cursor is moved to all possible selections; press "EXIT" to return immediately.

NOTE: The changes made remain in effect as part of the parameters stored in memory for *this and all* programs.

Check Diagnostics

The Diagnostics screen can be displayed by selecting "Check Diagnostics" from the Stop Menu. This allows the user to determine what errors may have occurred and, with certain errors, to correct the condition causing the error before processing is delayed further. Refer to Section 7, TROUBLE-SHOOTING, for complete information on the displayed errors. Press "EXIT" to return to the Stop Menu.

Check Paraffin

This routine allows the operator to override the automatic delay time that ensures the paraffin is completely melted. If the retort or oven has cooled down because the instrument was turned *off* or because of a power outage, automatic processing will be delayed until the appropriate temperature has been reached and maintained for a specified length of time, as outlined in Table 4-1. Once the appropriate temperature is reached, processing will begin (or continue) until a paraffin station is reached (if the power outage occurred at a station prior to Station 11). If the delay time has not expired, processing will be delayed until it has.

Verification that the paraffin is molten (e.g., if it is melted in another oven and then replaced into the VIP oven) will allow processing to continue in the paraffin stations. Select "Check Paraffin" from the Stop Menu and verify that the paraffin is completely melted in each of the four paraffin tanks. Press "YES" if it is, and the warning message of "Paraffin Not Melted" will disappear; press "EXIT" if it is not. The display will return to the Stop Menu with either response.

Retort Temperature – Decrease from Set Temperature	Additional Standby Time hours (′), minutes (″)	Oven Temperature – Decrease from Set Temperature	Additional Standby Time hours (′), minutes (″)
0 – 4° C	5″	0 – 10° C	0″
5 – 6° C	25″	11 – 12° C	1′00″
7 – 8° C	40″	13 – 14° C	3′30″
9 – 10° C	1′00″	15 – 16° C	6′00″
11 – 12° C	2′10″	17 – 18° C	7′00″
13 – 14° C	4′00″	19° C or more	24′00″
15 – 16° C	5′30″		
17 – 18° C	7′00″		
19° C or more	24′00″		

Table 4-1

Discontinue Processing

The processing run can be discontinued by selecting this option. The message line will prompt "`Discontinue processing? Are you sure? Press ON/YES or OFF/CL.`" A response of OFF/CL returns the display to the Stop Menu with the original message line prompt of "`Select menu number. Press ENTER. Press START to resume processing.`" Responding YES will end the processing run and return the display to the Main Menu. If the retort is not empty, the message "`Retort is not empty.`" will be flashed on the Warning line and the alarm will sound for 10 seconds (repeating every minute). To silence the alarm, press "OFF."

4. Unless the process was discontinued, it can be restarted simply by pressing "START" from the Stop Menu. Processing will continue from the point at which it was interrupted. The instrument does NOT adjust the Remain Time for the length of time that elapsed while the instrument was stopped.

Recommendations for Efficient Operation

Specimen Orientation

Proper placement of tissue specimens into the basket is vital for acceptable processing results. Improper placement can result in inadequate reagent penetration and in reagent cross-contamination. For optimal results:

1. Tissue samples must be small enough to allow sufficient clearance between the tissue and the cassette interior. This will ensure good flow of reagents on all sides of the tissue.
2. Use the vertical divider in the specimen basket.
3. Place cassettes in the basket with their slanted end facing up, rather than laying them flat.
4. Cover the baskets with their lids, latching them into place, to prevent cassettes from floating free in the retort.

Solution Replacement and Exchange

The schedule for replacement and/or exchange of reagents varies from one laboratory to another; the volume of processing is the best indicator of the timing. Rotation of reagents can be done either manually, by physically moving the bottles from one station to another, or automatically, through the "Exchange Solutions" Mode.

Paraffin Replacement

The paraffin tanks are designed to be removed from the oven for filling and should be filled with previously-melted paraffin only. (Placing unmelted paraffin flakes or chips in the tanks may cause the oven to overheat and trip a circuit breaker.) Carefully observe the fill line, and do NOT overfill. When transferring paraffin from one container to another, hook the lip of the pouring container over the edge of the receiving container. Pour molten paraffin slowly and carefully.

WARNING: Oven and paraffin containers are hot! Use extreme care.

Replacement of Fume Control System Materials

The water in the water bottle absorbs formalin and alcohol vapors from the exhaust fumes. Change the water daily to maintain efficient fume control.

The activated carbon in the carbon cartridge absorbs the remaining xylene and alcohol vapors present in the exhaust fumes. To maintain a high level of fume control, change the cartridge once a month (after 20 processing runs).

ACCESSORIES

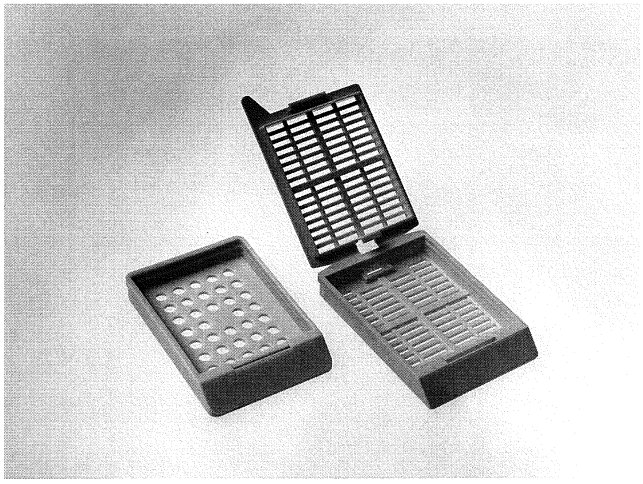


Figure 5-1

Tissue-Tek® Uni-Cassettes® Tissue-Tek® Process/ Embedding Cassettes

Tissue-Tek Cassettes (Figure 5-1) are designed for use with various fixative, clearing, and dehydrating reagents. The Uni-Cassettes are self-contained units with an attached lid that is removed prior to embedding with paraffin. The Process/Embedding Cassettes are designed for use with reusable metal lids, such as Tissue-Tek Process Covers. Tissue-Tek Cassettes are available in a variety of colors and styles.

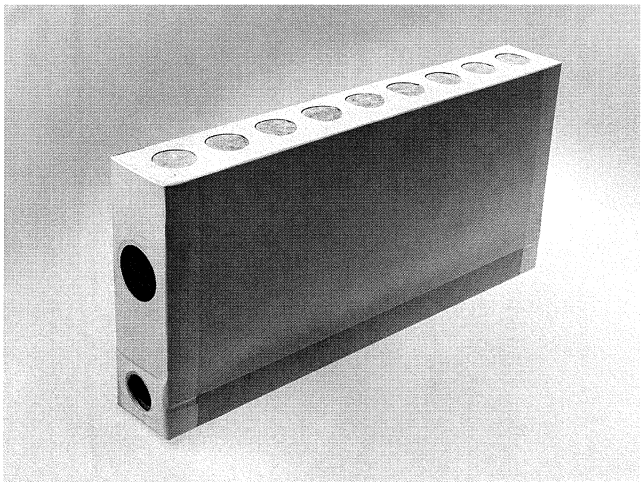


Figure 5-2

Tissue-Tek® VIP™ Activated Carbon Cartridge (Product No. 4899)

The Tissue-Tek VIP Activated Carbon Cartridge (Figure 5-2) is made specifically for use with the Tissue-Tek VIP Vacuum Infiltration Processor. It is self-contained for easy insertion and disposal.

CARE OF THE INSTRUMENT

General Maintenance

Keep the exterior of the instrument free of dust. Remove hardened paraffin using a plastic scraper. If needed, gently clean the remaining paraffin with a cloth *lightly* moistened with xylene, then wipe with a dry cloth. Do not use solvents of any kind on the front of the control panel; however, if disinfection of the keypad is desired, 70 to 85% ethanol or isopropanol can be wiped on and allowed to air dry. The glass doors may be cleaned with a glass cleaner and soft cloth. Keep the inside of the reagent cabinet clean by wiping with a clean cloth.

Daily Maintenance

Retort

1. Clean the inside of the retort lid, the rim of the retort, and the retort lid gasket by wiping with a clean cloth. After cleaning, inspect the gasket carefully for cracks, splitting, tearing, or other signs of deterioration that could cause leaks. Replace the gasket if necessary, as instructed in Section 8, MINOR REPAIR.
2. Clean the inlet/outlet filter in the retort floor, as follows:
   a. Use a large flathead screwdriver to loosen the screw in the center of the filter (Figure 6-1), then remove the filter and screw from the retort (the screw and filter will remain together).

Figure 6-1

   b. Remove any debris, then thoroughly clean the filter by soaking in xylene. Clean the retort floor by wiping with a cloth moistened with xylene. Reinstall the filter, tightening the screw gently with the screwdriver.

Fume Control System — Water Bottle

Remove the WATER bottle from the storage cabinet and dispose of its contents. Refill to the proper level (2.2 L) with tap water and replace into the reagent cabinet.

Exterior Surfaces

Daily clean the control panel, plastic, and painted areas by wiping with a clean cloth moistened with mineral oil. Daily clean the inside of the reservoir storage compartment by wiping with a clean cloth.

Weekly Maintenance

Reagent Exchange and Warm Water Flush

1. All the reagents should be replaced or exchanged on a weekly basis. If replacing, empty each bottle and refill it with the appropriate concentration of fresh reagent. If exchanging, empty the first bottle of each reagent type, then move the remaining reagents up one station each (see "Exchange Solutions" in Section 3).
2. The formalin (generally in Stations 1 and 2) and the first alcohol (generally Station 3) become contaminated with precipitated formalin salts. These salts also create blockages in the lines and in the rotary valve. Therefore, the formalins and the first alcohol should be replaced, rather than exchanged, on a weekly basis. While replacing these reagents, a warm water flush should be performed to dissolve any salt buildup in the lines and on the rotary valve:
   a. Discard the contents of each bottle of formalin and the first bottle of alcohol (generally Stations 1-3). Rinse each bottle with tap water, then fill each to the appropriate line with warm tap water. Reinstall the bottles into the appropriate stations.
   b. Pump the water into and out of the retort at each station, either through the Manual Mode or automatically using a specially created program:

      MANUALLY: From the Main Menu, select "9. Manual Mode," then select "3. Move Station" as often as necessary to move the rotary valve to Station #1. Select "2. Pump In" to pump the water from Station 1 into the retort. When the pump-in is complete, select "1. Pump Out" to pump the water back into the bottle. Move the rotary valve to the next station and repeat the pump-in and pump-out. Repeat for each appropriate station.

      AUTOMATICALLY: Create a program for the procedure through the "Edit Programs" Routine, using a free program. Specify a processing time of 1 minute ("0:01") for each of the formalin stations and the first alcohol station; specify zero time ("0:00") for all remaining stations. Perform a processing run using this program number and Immediate start mode. When the cycle is complete, bypass the Clean Cycle by pressing "EXIT" and entering the password. **Note:** When creating an automatic program for the warm water flush, do *not* edit the solution codes to indicate water in the first several stations; doing so will change the codes for *all* programs.

   c. When water has been pumped from all appropriate stations, discard the water from each bottle and fill with the appropriate concentration of fresh reagent. Replace the bottles into their appropriate stations, ensuring a secure connection.

Clean Cycle Reagents (Stations 15 and 16)

The xylene and alcohol used in the Clean Cycle will quickly become contaminated with residual paraffin; therefore, these reagents need to be replaced after every five uses. (If local regulations require the use of a xylene substitute, it should be replaced more often; also, extra cleaning cycles may be needed to attain proper cleaning.) Discard the contents of each bottle in an appropriate manner, then fill with fresh reagent to the appropriate "C" line. Reinstall the bottles into their proper stations, ensuring a secure connection.

Spill Trays

WARNING: The spill trays may contain flammable liquid. Handle with care.

Carefully remove both spill trays (one under the paraffin oven, the other under the reagent cabinet). Dispose of any liquid waste from the reagent tray by draining into an appropriate container, then wipe with a clean cloth. Remove any paraffin accumulation from the oven tray with a scraper, then clean thoroughly by wiping with a xylene-moistened cloth. Reinstall the trays into their proper locations.

Periodic Maintenance

Fume Control System — Activated Carbon Cartridge

Replace the activated carbon cartridge on a regular basis. The recommended schedule is once a month (assuming five processing runs per week); however, this will vary depending upon the frequency and duration of your runs and on the reagents used. Remove the old cartridge from the reagent cabinet (you may need to remove the WATER bottle in order to grasp the cartridge) and replace with a new cartridge (Product No. 4899). Press the cartridge firmly into place to ensure a proper seal. Dispose of the used cartridge according to your local regulations.

CAUTION: The used carbon cartridge contains xylene fumes; dispose of these cartridges with proper care.

Overflow Bottle

The overflow bottle, located behind the rear panel on the control module, should be inspected periodically (once or twice a year) for fluid buildup, by a qualified service person.

Bottle Caps and O-Rings

Carefully inspect all bottle caps, on both ends of each bottle, for cracks and worn threads. Inspect the O-rings for cracks and deterioration (the ring for the small front cap is inside the cap; the ring for the large rear cap is on the neck of the bottle). An initial indication of a cap or O-ring problem will be the loss of bubbling in the WATER bottle during fluid transfer operations. Replace caps or O-rings that are cracked or deteriorated (see Section 9, "Replacement Parts").

Disinfection

Under normal operating conditions, the instrument is effectively disinfected each time the Clean Cycle is performed. However, if a malfunction of the pump occurs and normal pumping cycles cannot be accomplished, the instrument can be disinfected, if necessary, by the following procedure:

1. Spray or wipe a thick coating of 70 to 85% ethanol or isopropanol onto the walls and base of the retort and the inside of the retort lid. Do not use absolute (100%) alcohol, as this is a less effective disinfectant than is a concentration of 70 to 85%.

   NOTE: The use of sodium hypochlorite (i.e., bleach) is *not* recommended, since any residue in the retort can contaminate the reagents and tissues.

2. Allow the alcohol to air dry before closing the retort lid.

TROUBLESHOOTING

General Information

The following Troubleshooting Chart lists the warning messages and error codes that could occur during operation of the Tissue-Tek® VIP™ instrument. Possible problems could be electrical, mechanical, or operational. Probable causes and recommended remedies are also included, so that many isolated problems can be quickly corrected.

If additional assistance is required concerning an instrument problem, or if the problem cannot be isolated or is beyond the scope of this manual, complete the "Preservice Checklist" in Section 9. Then contact the Customer Support Department of Sakura Finetek U.S.A., Inc., by calling toll free 1-800-725-8723 (U.S. only). If located outside the United States, contact the nearest Tissue-Tek instrument distributor or representative for information and assistance.

When an error is detected, an alarm sounds for about 10 seconds, repeating every minute until error is cleared. The error number or warning message is flashed repeatedly on the "Warning" line of the display. If the instrument is left unattended for longer than five minutes, an external alarm, if connected, is activated. To stop the internal alarm, press the "OFF/CL" key. To turn off the external alarm, slide the retort bar to the left and then to the right.

TROUBLESHOOTING CHART

WARNING MESSAGE	DESCRIPTION	REMEDY
Battery voltage low	All user-selected programming, such as reagents and programs, are maintained by the backup battery if a power outage occurs. If power is lost and the battery voltage is insufficient to provide this backup, then all user-selected programming will be lost.	Contact Customer Support to have a new battery installed. Do not turn your instrument *off* until the battery is replaced to ensure that your programming remains in memory.
Paraffin not melted	Displayed when the instrument is first turned *on* or when there has been a power outage in which the oven temperature has dropped by greater than 10° from the set temperature. Will continue to be displayed for a preset length of time, up to 24 hours.	When the oven temperature reaches its set temperature, this warning can be overridden through the Main Menu option of "Check Paraffin" (see Section 3). Ensure that the paraffin in each tank is completely melted before overriding the warning.
Power out	Power to the instrument has been interrupted because of a power outage, the power switch was turned to OFF, or the line cord was unplugged.	Power has already been restored when this warning is displayed. Operation continues immediately unless the oven or retort temperatures have fallen below the acceptable limit, in which case an additional delay may occur. (See "Paraffin not melted" above.) The end time for the processing run may be (or have been) delayed. To clear the warning from the display, slide the retort bar to the left and then to the right.
Retort dirty, Clean retort	The retort has not yet been cleaned following a processing run in which reagent from Stations 11-14 was pumped in.	Perform a Clean Cycle by selecting "8" from the Main Menu.
Retort is not empty	Processing was discontinued while there was reagent in the retort.	Drain the retort by selecting "11" from the Main Menu.
Retort lid unlocked	The retort bar is in the "open" position (to the left). No processing operation (automatic or manual) will occur unless the retort bar is closed. If the bar is opened while the instrument is operating, an alarm will sound for ten seconds, repeating every minute.	Be sure the retort lid latches are securely closed, then slide the retort bar to the right.

ERROR CODE	DESCRIPTION	POSSIBLE CAUSE	REMEDY
1	Retort overflow during a P/V cycle	1. A reagent bottle contains too much liquid.	1. Fill bottles to the correct level for the instrument model being used.
		2. There are too many specimens in the retort.	2. Reduce the number of specimens in the retort; do not process more than the recommended number.
		3. Fluid from an external source was poured into the retort.	3. Open retort and remove excess fluid to below the fill line (etched on either side of retort), then close retort. Place an empty bottle at the highlighted station and perform a manual pump-out of the retort.
		4. Overflow bottle is misadjusted or overflow sensor was touched or is misadjusted.	4. Perform a manual pump-out. If error repeats, contact Customer Support for further information.
2	Retort overflow during other than a P/V cycle or pump-in	1. The instrument was moved while fluid was in the retort, causing a "false" overflow.	1. Do not move the instrument when there is fluid in the retort.
		2. Overflow bottle is misadjusted or overflow sensor was touched or is misadjusted.	2. Perform a manual pump-out. If error repeats, contact Customer Support for further information.
3	Power outage	There was a power outage at some point during the processing cycle.	Refer to "Power Out" warning above. Before continuing the run, check that the paraffin is molten after the retort and oven temperatures have stabilized.
10	Retort overflow during the pump-in cycle	1. A reagent bottle contains too much liquid.	1. Perform a manual pump-out, then fill all bottles to the correct level for the instrument model being used.
		2. There are too many specimens in the retort.	2. Reduce the number of specimens in the retort; do not process more than the recommended number.
		3. Fluid from an external source was poured into the retort.	3. Open retort and remove excess fluid to below the fill line (etched on either side of retort), then close retort. Place an empty bottle at the highlighted station and perform a manual pump-out of the retort.
11	Pump-in not complete after 6 minutes	1. Reagent bottle is not securely connected to quick-release connector.	1. Reconnect the bottle at the station listed, ensuring a secure connection of the quick-release connector to the bottle.
		2. Air line or reagent transfer line is plugged.	2. Contact Customer Support for further information.

ERROR CODE	DESCRIPTION	POSSIBLE CAUSE	REMEDY
12	Pump-in not complete after 3 attempts of 6 minutes each	Refer to Error #11.	Refer to Error #11.
13	Unable to establish pressure or vacuum inside the retort	1. Retort latches are loose.	1. Tighten the latches by turning clockwise (refer to Section 2, "Instrument Setup").
		2. Retort gasket is leaking.	2. Clean and reposition the retort gasket, as instructed in Section 6. If gasket appears cracked or worn, replace as instructed in Section 8.
		3. A line connection or union is loose.	3. Contact Customer Support for further information.
		4. Instrument malfunction.	4. Contact Customer Support.
14	Unable to establish vacuum from reagent bottle	1. Reagent bottle is empty.	1. Fill the bottle with the appropriate reagent to the proper fill line.
		2. Paraffin tank (Stations 11-14) is not securely connected.	2. Reconnect the tank at the station listed, ensuring a secure connection at the back of the tank.
		3. Instrument malfunction.	3. Contact Customer Support.
20	Pressure cannot be achieved in 1 minute with the rotary valve closed	Refer to Error #13.	Refer to Error #13.
21	Pressure cannot be achieved after 3 attempts of 1 minute each with the rotary valve closed	Refer to Error #13.	Refer to Error #13.
22	Pump-out not complete after 2 attempts of 6 minutes each	1. Reagent bottle or paraffin tank is not securely connected.	1. Reconnect the bottle or tank at the station listed, ensuring a secure connection.
		2. Retort inlet/outlet filter is plugged.	2. Clean the filter as instructed in Section 6, "Daily Maintenance."
		3. Air line or reagent transfer line is plugged.	3. Contact Customer Support for further information.
23	Pump-out not complete after 2 attempts of 6 minutes each	Refer to Error #22.	Refer to Error #22.
31	Oven thermistor malfunction (Connector #9)	Connector is not connected; the connector pin has come off; or the thermistor wire is disconnected.	Contact Customer Support for further information.
32	Rotary valve thermistor malfunction (Connector #11)	Refer to Error #31.	Refer to Error #31.
33	Retort thermistor malfunction (Connector #10)	Refer to Error #31.	Refer to Error #31.

ERROR CODE	DESCRIPTION	POSSIBLE CAUSE	REMEDY
34	Oven temperature too high	The thermistor circuit has been tripped.	Contact Customer Support for further information.
35	Oven temperature too low	Refer to Error #34.	Refer to Error #34.
36	Rotary valve temperature too high	Refer to Error #34.	Refer to Error #34.
37	Rotary valve temperature too low	Refer to Error #34.	Refer to Error #34.
38	Retort temperature too high	Refer to Error #34.	Refer to Error #34.
39	Retort temperature too low	Refer to Error #34.	Refer to Error #34.
40 to 88	Instrument malfunction	1. A strong external source of light is interfering with the photo interrupter in the instrument.	1. Turn *off,* or move the instrument away from, any source of very bright light that shines into the rear of the instrument. (Overhead lights will generally not affect the instrument.)
		2. Instrument malfunction.	2. If Step #1 does not correct the occurrence of the error, contact Customer Support.

MINOR REPAIR

General Information

This section is provided as an aid for performing minor repairs on the Tissue-Tek® VIP™ instrument. The only procedure that can be performed without direct interaction with a Customer Support Representative is the replacement of the retort gasket. For any repairs other than the one given in this section, refer to Section 9, SERVICE AND REPLACEMENT PARTS, for instructions on service for your instrument.

Replacement of Retort Gasket

If the retort gasket is worn or cracked, the instrument will be unable to maintain pressure or vacuum in the retort. The gasket can be replaced by the following procedure.

Tools Required:

None

Parts Required:

Retort Gasket (Part No. 1520)

Procedure:

1. Remove the old gasket from the groove around the underside of the retort lid.
2. Thoroughly clean the groove using a cloth moistened with alcohol or xylene. Be sure all debris and moisture are removed. Then check the groove carefully for any flaw or irregularity. If any exists, do not operate the instrument; contact Customer Support immediately.
3. Replace the new gasket into the groove. The wider side of the gasket goes into the groove, with the narrower side facing out (see Figure 8-1).

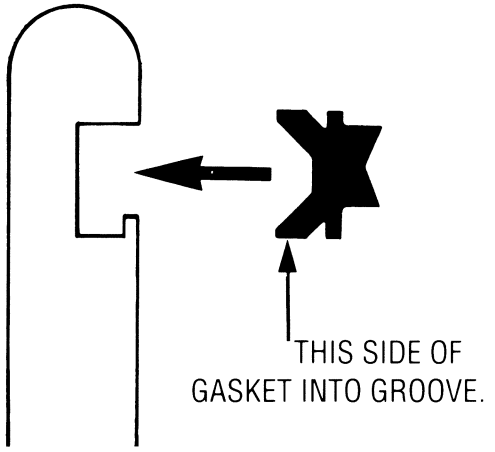


Figure 8-1

Once a small portion of the gasket is fitted into the groove, slowly run your thumb along the gasket, pressing it into the groove all the way around the lid (Figure 8-2). Check carefully to ensure that the entire length is fully inserted inside the groove, with no edges sticking out.

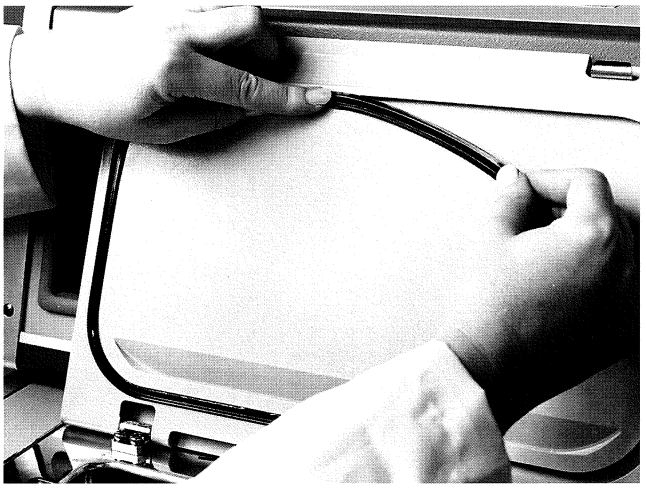


Figure 8-2

SERVICE AND REPLACEMENT PARTS

Service Information

When You Have a Problem With the Instrument

When problems arise during operation of the Tissue-Tek® VIP™ Vacuum Infiltration Processor, first refer to Section 7, TROUBLESHOOTING. Avoid problems by carefully following proper operating and cleaning procedures. If the problem cannot be solved and an instrument failure is apparent, our Customer Support Department is available to assist you.

Before calling for instrument service, collect the information requested in the "Preservice Checklist" in this section. This information will help the Customer Support Representative to identify the probable cause of your instrument malfunction.

The instrument should be disinfected prior to being serviced. If the pump is functioning, performing a Clean Cycle will provide sufficient disinfection. If it is not functioning, the retort should be disinfected as described in Section 6, CARE OF THE INSTRUMENT.

Where to Call for Service

If located within the United States, contact the Customer Support Department of Sakura Finetek U.S.A., Inc. by calling toll free:

1-800-725-8723

In countries other than the United States, contact the nearest authorized Tissue-Tek instrument distributor or representative for service information and assistance.

Tissue-Tek VIP
Preservice Checklist

For reference, record the following information:

Model / Serial Number: ___ ___ ___ ___ – ___ ___ ___ ___ ___ ___ Installation Date: _____

1. Has Section 7, Troubleshooting, been reviewed? ☐ YES ☐ NO
2. When the power is turned *on,* does the screen show an appropriate display? ☐ YES ☐ NO
   - If NO, is the unit plugged into a live AC electrical outlet? ☐ YES ☐ NO
3. Are all reagent bottles securely connected to the quick-release connectors? ☐ YES ☐ NO
4. Are all reagent bottles filled to their proper levels? ☐ YES ☐ NO
5. Are any warnings being displayed? ☐ YES ☐ NO
   - If so, have they all been corrected and cleared? ☐ YES ☐ NO
   - If you are unable to clear a warning, what does it say?

     _____

     _____
6. Are any errors listed in the "Check Diagnostics" screen? ☐ YES ☐ NO
   - If so, please list the information from that screen:

Error	Sta.	Retort Temp.	Oven Temp.	Rotary Valve Temp.	Time

7. Do the oven and retort temperatures regulate properly? ☐ YES ☐ NO

   Oven Temperature: _____° C Retort Temperature: _____° C
8. Which station number is being highlighted? _____
9. Is an "Operation" being displayed? ☐ YES ☐ NO
   - If YES, which?
     Pump In ☐ Pump Out ☐ Move Station ☐
10. Does the "Retort" display show an operation? ☐ YES ☐ NO
    - If YES, which?
      Vacuum ☐ Ambient ☐ Pressure ☐
11. Is there fluid in the retort? ☐ YES ☐ NO
    - If YES, how much? **CAUTION: Do NOT attempt to open the retort if the display shows either "Vacuum" or "Pressure."** _____
12. Does the pump operate? ☐ YES ☐ NO
13. What is the level of vacuum and/or pressure on the pressure gauge (visible through the rear panel behind the reagent cabinet)?

    Pressure: _____kg/cm^2 Vacuum: _____cm Hg
14. Are the time and date being displayed accurately? ☐ YES ☐ NO

Replacement Accessory Items

Product No.	Description
4899	Tissue-Tek® VIP™ Activated Carbon (2 Cartridges)
4397	Printer

Replacement Parts

Product No.	Description
1520	Retort Gasket
1521	Cassette Basket, Lid, and Divider
1522	Reagent Bottle (w/o Caps & O-Rings)
1523	Reagent Bottle Cap, rear
1524	Reagent Bottle O-Ring, rear
1525	Reagent Bottle Cap, front (with O-Ring)
1526	Reagent Bottle (Complete)
1550	Paraffin Scraper
060-334-00	Spill Tray
064-329-01	Paraffin Tank, Models E150
065-329-01	Paraffin Tank, Models E300
B4-00-0230	Overflow Bottle
200-7980	Printer Interface Kit
200-7990	U.P.S. Interface Kit
999-489-08	Service Manual
999-489-09	Operating Manual

Where to Order:

In the United States, the above Replacement Accessory Items and Replacement Parts may be ordered directly from:

ORDER SERVICES
Sakura Finetek U.S.A., Inc.
18700 Crenshaw Blvd.
Torrance, CA 90504

or by calling toll free:
1-800-725-8723

Outside of the United States, contact the nearest authorized Tissue-Tek instrument distributor.

EXAMPLE PROCESSING PROGRAMS

Sample Programs

Following are two examples of programs, one for a routine overnight run and the other for a rush or biopsy run. Example reagents and concentrations are shown also. The programs are intended to be used as a guide only; modify them to satisfy the individual needs of your laboratory. Following the example programs are several pages on which you can write your own individual programs.

EXAMPLE PROCESSING PROGRAMS (To be used as a guide only)

Routine Overnight Run

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1	Neutral Buffered Formalin	05	10%	2:00	40°	On	On
2	Neutral Buffered Formalin	05	10%	2:00	40°	On	On
3	Ethanol	12	65%	0:30	40°	On	On
4	Ethanol	12	80%	0:30	40°	On	On
5	Ethanol	12	95%	0:45	40°	On	On
6	Ethanol	12	95%	0:45	40°	On	On
7	Ethanol	12	100%	0:45	40°	On	On
8	Ethanol	12	100%	0:45	40°	On	On
9	Xylene	20	100%	0:45	40°	On	On
10	Xylene	20	100%	0:45	40°	On	On
11	VIP Paraffin	30		0:30	58°	On	On
12	VIP Paraffin	30		0:30	58°	On	On
13	VIP Paraffin	30		0:30	58°	On	On
14	VIP Paraffin	30		0:30	58°	On	On

Rush or Biopsy Run

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1	Neutral Buffered Formalin	05	10%	0:30	40°	On	Off
2	Neutral Buffered Formalin	05	10%	0:30	40°	On	Off
3	Ethanol	12	65%	0:10	40°	On	Off
4	Ethanol	12	80%	0:10	40°	On	Off
5	Ethanol	12	95%	0:10	40°	On	Off
6	Ethanol	12	95%	0:15	40°	On	Off
7	Ethanol	12	100%	0:15	40°	On	Off
8	Ethanol	12	100%	0:15	40°	On	Off
9	Xylene	20	100%	0:15	—	On	Off
10	Xylene	20	100%	0:15	—	On	Off
11	VIP Paraffin	30		0:15	58°	On	Off
12	VIP Paraffin	30		0:15	58°	On	Off
13	VIP Paraffin	30		0:15	58°	On	Off
14	VIP Paraffin	30		0:15	58°	On	Off

PROGRAM 1

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 2

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 3

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 4

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 5

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 6

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 7

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 8

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

PROGRAM 9

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

EXTRA PROGRAM

Station No.	Solution	Code No.	Conc.	Time (hr:min)	Set Temp.	P/V	Agit
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

Sakura Service Training Manual, VIP E Series
Operating Addendum Documents:

Page	Document Name	Author	Date
1-4	Tissue Tek VIP Reagent Compatibility	Bayer	
5	489_ Operations Test, 489Op Test	R. Heard	7/24/98
6-11	Overview of Histology	Dr. J. Cumminskey	

C. Johnson 489XAdend 10/28/99

TISSUE-TEK® V.I.P.™ REAGENT COMPATIBILITY

FIXATIVES - COMPATIBLE	MANUFACTURER/SUPPLIER	COMMENTS*
Buffered Formalin, up to 20%		
Unbuffered Formalin, up to 20%		
Alcoholic Formalin		
Millonigs Buffered Formalin		
Zinc Formalin		Zinc component
Alcoholic Zinc Formalin		Zinc component
Ultrum II	American Histology Reagent Co.	Zinc component
Z-Fix	Anatech	Zinc component
Pen-Fix™	Richard-Allan	
Formal Free	Stat Fix	
Forma-Scent®	Baxter	
Formalde-Fresh®	Fisher	
Omni-Fix	AnCon Genetics, Inc.	Formalin substitute
STF™	Streck	Formalin substitute
HistoChoice™	Amresco	Formalin substitute
Prefer	Anatech	Formalin substitute
No Tox	Earth Safe Industries	Formalin substitute
Normalin	S & S Company of Georgia	Formalin substitute
FIXATIVES - NOT RECOMMENDED	**MANUFACTURER/SUPPLIER**	**COMMENTS**
Bouins		Picric acid precipitate
B-5		Chloride concentration exceeds 1.0%
Zenker's		Chloride concentration exceeds 1.0%
Helly's		Chloride concentration exceeds 1.0%
Ultrum	American Histology Reagent Company	Chloride concentration exceeds 1.0%
Specifix	Decal Chemical Corporation	Chloride concentration exceeds 1.0%

*Recommended instrument maintenance, warm water flushes for all fixatives. Contact Customer Service 800-348-8100 for procedure.

TISSUE-TEK® V.I.P.™ REAGENT COMPATIBILITY

DEHYDRANTS - COMPATIBLE	MANUFACTURER/SUPPLIER	COMMENTS
Ethyl Alcohol		
Isopropyl Alcohol		
Methyl Alcohol		
Reagent Alcohol		
Butanol		
S-29	Miles	
Pro-Soft	Anatech	
Flex	Richard-Allan	
Dehydrant	Richard-Allan	
DEHYDRANTS - NOT RECOMMENDED	**MANUFACTURER/SUPPLIER**	**COMMENTS**
Acetone		Damages rubber components
Tissue Dry	Fisher	Damages rubber components

TISSUE-TEK® V.I.P.™ REAGENT COMPATIBILITY

CLEARANTS - COMPATIBLE	MANUFACTURER/SUPPLIER	COMMENTS
Xylene		
Toluene		
Benzene		
Chloroform		
Pro-Par	Anatech	Xylene substitute
Clear-Rite 3™	Richard-Allan	Xylene substitute
Hemo-De™	Fisher	Xylene substitute
Americlear®	Baxter	Xylene substitute
Parasol	Baxter	Xylene substitute
Histosolv-X	CMS	Xylene substitute
Safeclear	CMS	Xylene substitute
Slide Brite	S & S Company of Georgia	Xylene substitute
CLEARANTS - NOT RECOMMENDED	**MANUFACTURER/SUPPLIER**	**COMMENTS**
Clearene	Surgipath	Rotary valve failure
Tissue Clear II	Fisher	Rotary valve failure
Tissue Clear III	Fisher	Rotary valve failure

TISSUE-TEK® V.I.P.™ REAGENT COMPATIBILITY

PARAFFIN - COMPATIBLE	MANUFACTURER/SUPPLIER	COMMENTS
V.I.P.™ Paraffin	Miles	
Paraplast®		
Paraplast Plus		
Paraplast X-tra		
Tissue Prep®	Fisher	
Tissue Prep 2	Fisher	
Ameraffin®	Baxter	
Ameraffin LP	Baxter	
Infiltration Medium	Surgipath	
Blue Ribbon®	Surgipath	

489__ Operations Test
Questions from the operator's manual.

1. What are the five principles of operation during a process? (pg. 1.5)
   (1.)_____
   (2.)_____
   (3.)_____
   (4.)_____
   (5.)_____

2. What reagent is designated for station 15 ____ 16 ____. (pg. 1.3)

3. What level should the water bottle be filled to, regardless of model? 150__ 300__ C__ P__ (pg. 2.5)

4. What does the P and C marking on the bottle mean? P _____ C _____ (pg. 2.5)

5. What marking should reagent bottles 1 through 10 be filled to? ____ (pg. 2.5)

6. The quick-release connector is designed to be turned once for a quick removal or connection.
   True ____ False ____ (pg. 2.4)

7. Have tech point out various parts of the instrument using the operator's manual. (pg. 1.2-1.4)
   Printer ____
   External relay ____
   Paraffin container ____
   Paraffin Oven ____
   Quick release ____
   Retort ___
   Retort Gasket ____

8. The process of transferring fluid from a reagent bottle into the retort is called _____.

9. On the main menu, each number is associated with which menu item: (pg. 3.1)
   ____ Start process
   ____ Drain retort
   ____ Password
   ____ Date and time
   ____ Manual operation
   ____ Exchange solutions
   ____ Check paraffin
   ____ Service operations
   ____ Identify solutions
   ____ Check diagnostics
   ____ Start clean cycle
   ____ Edit programs

10. What is the default password? _____ (3.6)

11. What is the proper installation of the retort lid gasket? _____ (pg. 8.1)

OVERVIEW OF HISTOLOGY

By: Dr. Jean Cummiskey

INTRODUCTION

Histology is the microscopic study of the structure of tissues. Pathology is the study of disease. Pathologic histology, or histopathology, is the microscopic study of diseased tissue.

The histology technician is concerned with preparing slides from various sources which will be examined microscopically by the pathologist in order to make a diagnosis of disease.

Sources of Tissue Specimens:
1) Autopsy Room
2) Surgical Suite
3) Clinical Offices
4) Research Labs
5) Other

Living tissue, or fresh tissue, is generally not suitable for detailed microscopic examination and must first be prepared. There are a number of steps in the preparation of tissue for microscopic examination.

Upon receipt of the tissue from the surgical suite, the autopsy room or the clinician's office, the specimen is assigned an accession number. This is the identification number which remains with the tissue throughout the entire process and is finally transferred to the prepared slide in the final step where the tissue specimen and its identification number are permanently mated.

GROSSING

Prior to any processing, the tissue (or in some cases the whole organ) must be cut into smaller pieces, usually less than 5mm thick and of a size and shape that will fit on a 1" x 3" glass slide. This step is called "GROSSING". In this step, care must be taken to select out that portion of the tissue or organ which is representative of abnormalities or structural changes.

Next begins the series of chemical treatments which will suitably prepare the tissue for microscopic examination and thus interpretation and diagnosis.

FIXATION

The first step in tissue processing is called fixation. Fixation is the foundation of all good histological preparations and this step must be effective and complete. There are several functions of fixation.
They are:

1) To prevent autolysis and bacterial decomposition.
2) To harden the tissue sufficiently so that the shape and volume are preserved during subsequent steps in the processing procedure.
3) To preserve the various cell constituents in as life-like a manner as possible.
4) To render the tissue suitable for a wide range of staining procedures.

The ideal fixative must be rapid, must be suitable for a wide range (type) of tissue and

it must be compatible with numerous stain techniques. All the needs and objectives of fixation can't be met by any one single fixative, and so some compromises inevitably occur. The most commonly used general fixative is 10% buffered neutral formalin. This is a rapid fixing agent, it is compatible with a wide range of stains and can be used for a wide range of tissues. It hardens the tissue sufficiently but does not create excessive hardening or damage.

There are some general rules of tissue processing which are worth repeating:

1) The tissue must be placed in the fixative rapidly after circulation ceases.

2) The tissue specimens must be cut thin enough so that the fixative will penetrate the sample quickly. Fixation begins at the periphery and proceeds inward. Slow penetration allows time for post mortem autolysis to occur in the center.

3) Tissue specimens should be immersed in 10-30 times their own volume, to reduce the percentage of contamination of the fluid.

4) The choice of a fixing agent is determined by the purpose for which the tissue is to be stained or preserved.

5) The pH of fixatives vary, but in general satisfactory fixation occurs between pH 6 and 8.

6) Temperature can be important. Fixation is traditionally done at room temperature, but for electron microscopy and histochemistry the range may be lower, 0-4C. Since chemical reactions occur faster at elevated temperatures, some have argued that fixation should be done at gradually increasing temperatures. Occasionally, the urgent fixation of biopsy material will be done at 60C but the risk of tissue distortion is increased.

The speed of fixation is of importance both to improve the work flow within the laboratory and to minimize the contact time between tissue and some fixatives which can overharden the tissue. A number of means are employed to increase the rate of fixation:

1. AGITATION: This insures contact between all surfaces of the tissue and the fixative and reduces the dilution of fixative in the immediate vicinity of the tissue by tissue fluids. Efficient agitation may reduce the fixation time by 25-30% with improved impregnation.

2. HEAT: The use of heat during fixation will hasten the process, but also causes shrinkage, brittleness and difficulties in sectioning and for that reason is not usually done. Also, some fixatives are flammable and cannot be left unattended during the application of heat.

3. SIZE OF TISSUE: Usually less than 5mm. When the tissue is contained within any type of capsule or cassette, the pieces of tissue must be trimmed to a significantly small size to allow freedom of movement of the block within the capsule. Poor processing results when the tissue is squeezed between the base and lid.

4. VACUUM: This can be used quite effectively during fixation and the later stage of infiltration with paraffin, but is probably of minimal value during dehydration and clearing.

5. NATURE OF THE FLUID ITSELF: Some fixatives increase the rate of penetration by increasing the permeability of the cell membrane. Other fixatives penetrate more or less rapidly depending on their viscosity; the higher the viscosity, the slower the rate.

6. ULTRASONICS: This has been suggested, but has not become a widely used technique.

7. MICROWAVE: This is perhaps the newest technique to be employed in reducing fixation time. It has been demonstrated to be quite effective if conditions of time and temperature are carefully controlled.

GENERAL PURPOSE FIXATIVES: 10% Buffered neutral formalin, the best overall fixative.

Ethyl Alcohol 70% - 100%: Slow penetration. Hardens and shrinks tissue. Best used for the preservation of glycogen.

Zenkers Solution: Post-fixation with potassium dichromate is required. Tissues must be well rinsed in running water (12 hours) before additional processing.

Bouin's Solution: Tissue must be well washed in 50% alcohol to remove picric acid. Tissues cannot be stored in Bouins for long periods of time.

Carnoy's Solution: Penetrates rapidly and no washing necessary. Disadvantages: Hemolyzes red blood cells.

Helly's Solution: Especially good of mitochondria.

DEHYDRATION

Once the tissue has been completely fixed in an appropriate fixing solution under compatible conditions, the free water in the tissue must be removed. This is necessary only if the tissue will ultimately be embedded in a non-aqueous medium such as paraffin. If the tissue will finally be embedded in a matrix such as carbowax, which is miscible with water, dehydration is not necessary.

There are a number of dehydrating agents available, but routinely dehydration is facilitated by bringing the tissue through a succession of alcohols of increasing strength, usually ranging from 70% to absolute (100%) alcohol. The tissue should remain in each strength for just as long as is necessary for complete saturation as prolonged immersion in the higher grades of alcohol will result in excessive shrinkage and hardening of the tissue.

In addition to graded alcohol, other dehydrating agents include:

Acetone — Rapid; quite flammable Dioxane — Very toxic fumes; miscible with paraffin

Tetrahydrofuran — Toxic, rapid, miscible with paraffin

Ethylene Glycol Monoethyl Ether (Cellosolve) — Rapid; Triethyl phosphate

CLEARING

Even though the tissue has now been dehydrated, the dehydrating agent may not be miscible with the impregnating medium such as paraffin and it is necessary to "clear" or "dealcoholize" the tissue. Both dehydration and clearing are steps designed to bridge the gap between an incompatible fixative and embedding medium. This most commonly occurs when an aqueous solution is used for fixation and paraffin is used for embedding. When we mentioned other dehydrating agents, dioxane and tetrahydrofuran are both miscible with paraffin and clearing agent is not necessary in that series.

The essential requirement of a clearing agent is that it be miscible with both the dehydrating agent and the embedding medium. There are many fluids which will fill this purpose, but only a limited number are in regular use. Most clearing agents are flammable which warrant caution, particularly when tissues are left unattended on automatic processing machines.

Some commonly used clearing agents are:
Xylene
Toluene
Chloroform
Benzene
Carbon Tetrachloride
Carbon Disulfide
Amyl Acetate
Cedarwood Oil

INFILTRATION

The next step is known as infiltration or impregnation. Since the fixed tissues are not firm enough or adhesive enough to permit sectioning without some supporting medium to hold the cells and intracellular spaces in proper relation to each other, it is necessary to support the tissue in a matrix. The most commonly used supporting medium is paraffin. Thus, the purpose of the infiltration step is to completely remove the clearing agent by substitution and replace it with the embedding medium. Vacuum applied during infiltration will hasten the removal of any air, gas and remaining clearing agent by vaporization and aid in drawing the paraffin into the tissues.

Most laboratories use paraffin with a melting point of 56-58C. Close control of the temperature is important since more than 5 above the melting temperature will cause excessive shrinkage and hardening.

While paraffin is the most commonly used material for infiltration and embedding, other materials are sometimes used:

Celloidin: Frequently used in central nervous system techniques

Plastics: Acrylic - Good for undecalcified bone

Epoxy: Used for electron microscopy

Gellatin: Low melting point so adverse effect of heat is avoided. Water soluble so dehydration and clearing are avoided.

Carbowax: Water soluble so sections can't be floated in water bath, but eliminates need for dehydration and clearing.

EMBEDDING

After the specimen is infiltrated with the embedding medium, it is encased in a solid matrix of the same material so that uniform sectioning is possible. The embedding material must convert easily from a liquid to a solid to permit both infiltration and sectioning.

With paraffin the conversion to a solid form is due to crystallization which is effected by a temperature reduction. With celloidin, the solid form is achieved by evaporation. When using plastics for embedding, polymerization occurs due to the action of either heat or a catalyst.

The rapid cooling of paraffin is recommended since this yields fine crystals, which provide better support of the tissue. Slow cooling results in large crystal formation which is unsatisfactory.

SECTIONING

The embedded tissue is now ready for sectioning. The most commonly used instrument in routine and research establishments is the Rotary Microtome, where the block holder moves up and down in front of the stationary knife. Modified models are available for cutting ultra-thin sections and for use in a CRYOSTAT for preparing frozen sections.

Objectives in cutting sections include:

1) Thin sections 4-6 microns
2) Good ribbons where the trailing paraffin edge of the front section adheres to the leading paraffin edge of the following section
3) Sections of uniform thickness
4) Unwrinkled
5) No scratches or tears in the tissue

Meeting these objectives require skill and experience on the part of the operator. Also required are a well-maintained Microtome, a sharp knife and properly prepared blocks.

The ribbons are transferred to a warm water bath where air bubbles and wrinkles can be teased out. A good representative section is selected, separated from the remaining sections, and floated onto a glass slide. The slide is immediately dried on a

hot plate, a drying oven or a slide dryer. Although thorough drying is necessary to bring about good adhesion of the specimen to the slide again, over-exposure to heat should be avoided as it causes distortion and poor staining.

FROZEN SECTIONS

While paraffin-embedded sections are the most commonly cut tissues, it should be mentioned that frozen sections also play an important role in histology. In preparing frozen sections, the water in the tissue is frozen to provide the supporting matrix. Additional support may be provided by such media as Lab-Tek's OCT Compound. Frozen sections are either cut from fixed or unfixed tissue. The common temperature for cutting is - 20C but this is varied to accommodate various tissues. In CRYOSTAT sectioning, the tissue is frozen rapidly (called snap frozen) in liquid nitrogen, alcohol/dry ice bath, aerosol sprays, or on the quick-freeze bar of the CRYOSTAT itself. After freezing, the issue is sectioned and picked up on a slide at room temperature and is ready for staining. Frozen sections are frequently fixed immediately after preparation and thawing although some procedures require the use of unfixed tissue.

Frozen sections are used for:

(1) the rapid diagnosis of stat specimens

(2) special procedures such as histochemistry or immunofluorescence, where the heat and chemicals employed in a typical tissue processing sequence would remove or denature the cell component of interest (enzymes, antigens, etc.)

(3) To permit the use of certain silver impregnation stains which cannot be used on paraffin embedded sections.

STAINS

The next step following sectioning is staining. The theory of staining is a very complicated issue and in some cases a poorly understood phenomena. Thus no further mention of theory will be made. Suffice it to say that while originally all dyes used for staining were natural products, today the most commonly used dyes with the exception of hematoxylin are synthetic products. Staining methods may be grouped into three general headings:

1) Vital staining which is applied to living tissue.

These stains may either be injected into some part of the animal body or mixed with living cells. This method is primarily a research tool.

2) Routine staining which is the basic "bread and butter" method for histology laboratories.

3) Special staining to demonstrate special features of the tissue such as bacteria, fungi, particular cell products and other inter and intracellular structures.

Hematoxylin and eosin is the most commonly used stain in histology. It will demonstrate an enormous number of tissue structures; it can be used with a wide variety of different tissues; it can be used with a wide variety of processing procedures; and is comparatively simple to use.

Essentially hematoxylin stains the cell nuclei blue-black with good intranuclear detail, and the eosin stains the cytoplasm and connective tissue in varying shades of pink-orange-red.

Hematoxylin is a natural dye and is extracted from the heartwood (logwood) tree which originated in Mexico but is now mainly cultivated in the West Indies. The hematoxylin itself is not a dye, but rather its oxidation product, hematein, is. The hematein can be produced from hematoxylin in two ways. One, the hematoxylin can be

exposed to light and air to facilitate "ripening". This is slow and can take from 3-4 months. A more rapid way of oxidizing or ripening the hematoxylin is to use a chemical oxidant, such as sodium iodate or mercuric oxide.

In addition, hematoxylin stains require the use of a mordant to increase its affinity for tissue. Alum is the most commonly used mordant.

The various hematoxylin stains in use today are either used as a progressive stain or a regressive stain. A progressive stain is one which is simply washed off when the right degree of staining is achieved and the counterstain is applied. A regressive stain is one which is initially overstained, and then the excess stain is removed with a decolorizer before the counterstain is applied. The Lab-Tek HISTO-TEK Stainer employs the regressive method of staining which employs Harris's hematoxylin, a chemically oxidized stain.

Another name for counterstain is secondary stain. Eosin is the most suitable one to combine with hematoxylin to demonstrate the general histological architecture of the cell. Hematoxylin and eosin staining is not only used on paraffin sections but can be used on frozen sections for stat work as well.

Prior to any staining procedures, paraffin sections must be deparaffinized and rehydrated. The deparaffinizing is done by soaking the tissue section on the slide in xylene and then backtracking through the graded alcohols to water.

In cases where cell constituents are not clearly differentiated by the hematoxylin and eosin stain, it is necessary to use a special stain. Some structures which are best visualized by special stains include elastic fibers, basement membrane, fibroblasts, mast cells, plasma cells, muscle, mucin, fibrin, calcium, mitochrondria, nucleic acids, etc.

MOUNTING

The final step in preparing the slide is to mount the specimen under a coverslip to facilitate handling and storage and to protect the tissue. There are two general types of mounting media which bond the coverslip to the slide-resinous and aqueous.

The resinous mountants are either natural or synthetic and are dissolved in such solvents as benzene, toluene or xylene. This means, of course, that the specimen which has just been stained in an aqueous dye solution must now be again dehydrated (usually in graded alcohols) and cleared in the mountant solvent, usually benzene, toluene or xylene.

Commonly used resin type mountants (and solvent) include:

Balsam Coverbond - Harleco's synthetic resin (xylene or benzene)

DPX - polystyrene resin (xylene)

Histoclad - Clay Adams synthetic resin (toluene)

Permount - Fisher Naphthalene (toluene)

Technicon resin - coumarone - indene polymer (benzene/xylene)

The aqueous mounting media are used when the stain will be discolorized or removed by the alcohol or xylene used for dehydration and clearing. After the aqueous based media have set, they can be permanently sealed by ringing the cover glass with clear nail polish.

THE END

SAKURA

Tissue-Tek® VIP™

Vacuum Infiltration Processor

E150/E300 Series

Printer Option

Operating Manual

SAKURA

Manufactured for:
Sakura Finetechnical Co., Ltd., Tokyo, 103, Japan
Sakura Finetek U.S.A., Inc., Torrance, CA 90504 USA
Sakura Finetek Europe B.V., Zoeterwoude, Netherlands
Made in U.S.A.

9996VPRI/Rev. 2/96

<< Chapter 1 >> Connecting Printer with VIP E-150 or E-300

Connect the printer by using the accessory printer interface cable.

<< Chapter 2 >> Operation

2-1 Prior to use

When power is supplied to the VIP E-150 or E-300, the power supply of the printer should also be turned on.

The printer should be set to "on line."

Confirm that paper for the printer is set.

2-2 Printer output

2-2-1 Out put of Program List and Solution List

After editing the Program List or when the edited Program List and Solution List are output, change to the Program Edit screen (**EDIT** mode).

The cursor can be positioned anywhere at that time.

Press the [*] and [v] keys. Shortly afterwards printout will begin.

[Sample of Output]

--- Program List ---

Jul. 24 1991

Instrument No. 0
--- Program No. 1 ---

Sta	Solution	Con.	Time	Temp.	P/V	Agit
1	Formalin	20%	1:30	C	Off	On
2	Formalin	20%	1:30	C	Off	On
3	M.Alcohol	50%	1:30	C	Off	On
4	M.Alcohol	60%	1:30	C	Off	On
5	M.Alcohol	70%	1:30	C	Off	On
6	M.Alcohol	80%	1:30	C	Off	On
7	M.Alcohol	90%	1:30	C	Off	On
8	Xylene	100%	1:30	C	Off	On
9	Xylene	100%	1:30	C	Off	On
10	Xylene	100%	1:30	C	Off	On
11	Paraffin	100%	1:30	60C	Off	On
12	Paraffin	100%	1:30	60C	Off	On
13	Paraffin	100%	1:30	60C	Off	On
14	Paraffin	100%	1:30	60C	Off	On

Endtime(date/hour:min) 1/ 9:00 am

[Solution]

Sta	Solution	Code	Con.	Set	Used
1	Formalin	1	20%	3	2
2	Formalin	1	20%	3	2
3	M.Alcohol	2	50%	4	2
4	M.Alcohol	2	60%	4	2
5	M.Alcohol	2	70%	4	2
6	M.Alcohol	2	80%	4	2
7	M.Alcohol	2	90%	4	2
8	Xylene	4	100%	5	2
9	Xylene	4	100%	5	2
10	Xylene	4	100%	5	2
11	Paraffin	9	100%	5	2
12	Paraffin	9	100%	5	2
13	Paraffin	9	100%	5	2
14	Paraffin	9	100%	5	2
15	Xylene	--	100%	5	2
16	E.Alcohol	--	100%	5	2
	A.Carbon		%	15	2

2-2-2 Output of processing result

A processing result is automatically output to the printer. If it is desired to output the previous processing result again, change the screen to the Main Menu screen (Main Menu mode).

The cursor can be positioned anywhere at that time.

Press the [*] and [V] keys. Shortly afterwards printout will begin.

Note: When an error occurs during the process, error information is also output at the same time.

[Sample of Output]

```
        --- Process Report ---
                                Jul. 24 1991

  Instrument No.          0
  Experiment No.   19910931
  --- Program No. 1 ---
 Sta Solution     Con. Time Temp. P/V Agit
 ------------------------------------------
  1  Formalin     20%  1:30     C  Off   On
  2  Formalin     20%  1:30     C  Off   On
  3  M.Alcohol    50%  1:30     C  Off   On
  4  M.Alcohol    60%  1:30     C  Off   On
  5  M.Alcohol    70%  1:30     C  Off   On
  6  M.Alcohol    80%  1:30     C  Off   On
  7  M.Alcohol    90%  1:30     C  Off   On
  8  Xylene      100%  1:30     C  Off   On
  9  Xylene      100%  1:30     C  Off   On
 10  Xylene      100%  1:30     C  Off   On
 11  Paraffin    100%  1:30   60C  Off   On
 12  Paraffin    100%  1:30   60C  Off   On
 13  Paraffin    100%  1:30   60C  Off   On
 14  Paraffin    100%  1:30   60C  Off   On
     Endtime(days/hour:min) 1/ 9:00 am

  ---
 Start Mode      Immediate

 Start Time     12:54 pm Jul. 23 1991
 End Time       10:34 am Jul. 24 1991
```

2-2-3 Output of error and alarm information

When the error and alarm information is output, change the screen to the error information screen (**Check Diagnostics** mode).

The cursor can be positioned anywhere at that time.

Press the [*] and [V] keys. Shortly afterwards printout will begin.

[Sample of Output]

```
      --- Diagnose ---
                                Jul. 24 1991

   Instrument No. 0

   [Diagnose]

  Error Sta  Retort  Oven  Valve   Time
     3    1     35C   62C    70C   2:42 pm


      --- Warning Report ---
  Power Out
```

2-3 When printout cannot be made

1) Confirm that the printer is correctly connected.

2) After confirmation that the printer is "on line", the printout can be made.

3) If the printer is in error or "off line" during printout, error number 90 is registered into the error information.

<< Chapter 3 >> Interface Specification

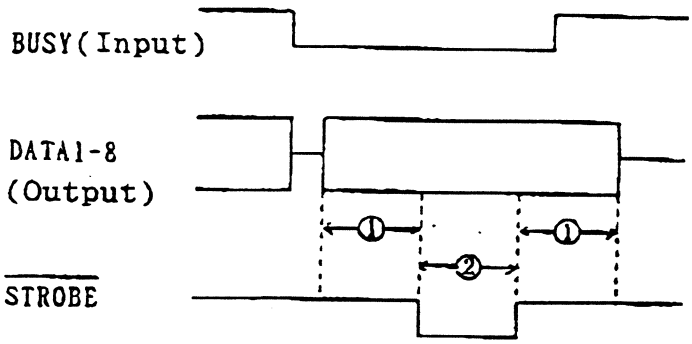
3-1 Connector pin positioning and timing chart

The printer of the VIP E-150 and E-300 refers to the Centronics specification. The 36 pin Centronics connector is used.

[Pin Positioning]

1	STROBE
2	DATA1
3	DATA2
4	DATA3
5	DATA4
6	DATA5
7	DATA6
8	DATA7
9	DATA8
10	未使用 (ACKNLG)
11	BUSY
12	PE
13～15	Not used
16	0V
17	FG
18	Not used
19～30	GND
31	INIT
32	Not used
33	GND
34～36	Not used

[Timing Chart]



① More than 1 μS and less than 5 μS.

② More than 1 μS and less than 5 μS.

Note: It is monitored by BUSY. The ACKNLG is not used.

<< Chapter 4 >> Precautions for use

1. Use the printer which refers to the Centronics interface.
2. Use the accessory cable or a similar one for the connection cable.
3. Use a printer which can print out more than 40 characters per line at a normal print setting.
4. Correspond the VIP E-150 or E-300 instrument to the character return (CR) of the printer.
5. Connect the VIP E-150 or E-300 instrument to the printer with the power supply turned off.
6. Confirm that the printer to be connected is functioning properly.
7. If the printer setting is changed to "off line" during output, subsequent printing may be canceled.
8. In regards to the other operations and functions, refer to the VIP E-150/E-300 Operating Manual.

** When the printer, Model CBM-530, is used **

When the printer CITIZEN CBM-530 is used, the following precautions should be taken:

1) If the power switch of the CBM-530 is turned on first and then, the power switch of the VIP E-150/E-300 is turned on, the buzzer will sound momentarily, but this is a normal occurrence.

2) If the power switch of the VIP E-150/E-300 is turned off before the power switch of the CBM-530 is turned off, the buzzer of the CBM-530 will sound. Turn off the power switch of the CBM-530.

** When a printer other than the CBM-530 is used **

Depending on the type of printer, one may have character return (CR) only and another may have character return and line feed (CR.LF). The VIP E-150/E-300 should use a printer with the character return and line feed (CR.LF). There are some printers which can be switched between the character return (CR) and the character return and line feed (CR.LF). If such is a case, select the character return and line feed (CR.LF) setting.

<< Appendix >>

WARNING	This page contains information on connecting printers other than the CBM-530. It is for use by service personnel only!

1. The following precautions need to be taken when printers other than the CBM-530 are connected.

   1) The printer must have a Centronics interface.

   2) The printer should be able to print more than 40 characters per line in a normal print setting.

   3) The VIP E-150/E-300 is preset to use a Type A printer, as shown below:

      Type A : Character return (CR) and line feed (LF)

      Type B : Character return (CR)
               Line feed (LF)

      When the printer is Type A, it can be connected as it is. Some printers can switch from Type A to Type B or vice versa by an internal switch. Refer to the printer operating manual for the printer setting.

      If the printer cannot be set to Type A, it will be required to change the internal switches of the VIP E-150/E-300.

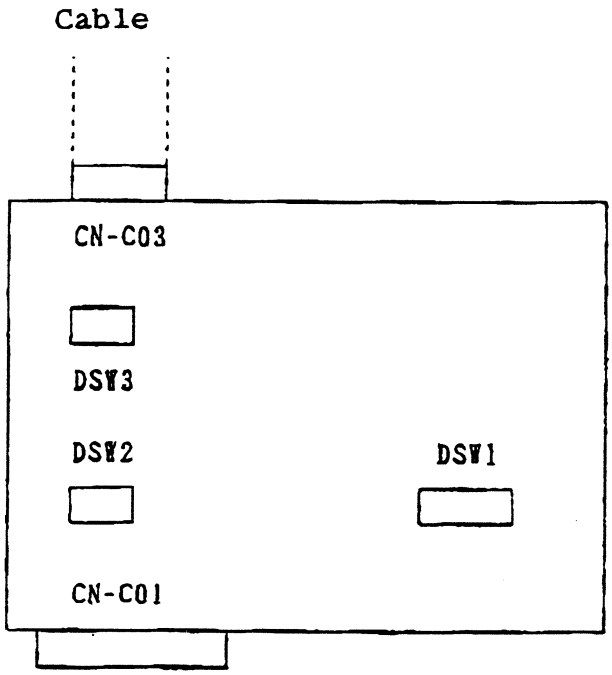
WARNING	This page contains information on connecting printers other than the CBM-530. It is for use by service personnel only!

2. VIP E-150/E-300 internal switch change procedure

The following operation should be performed after confirmation that the power switch has been turned off.

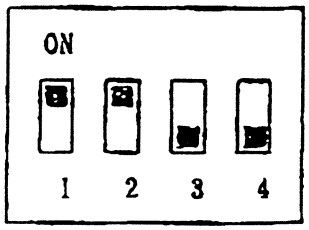
1) Remove the four (4) screws at the rear panel of the control box, and remove the outer casing.

2) There are two (2) PC boards in the PC board rack. The internal switches on the PC board at the rear panel side are changed as shown below:



Type A

D S W 3



Type B

D S W 3