Cytospin® 3 Cell Preparation System Operator Guide

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1. INSTALLATION AND SETTING UP

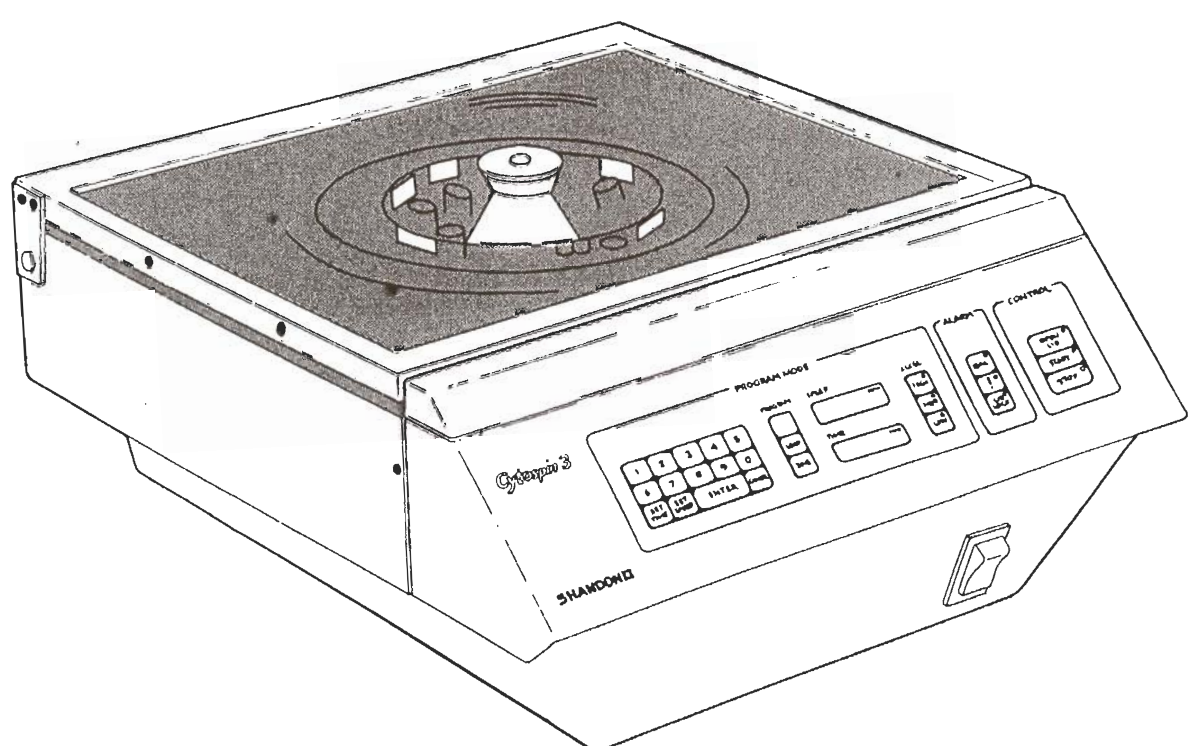
1.1 INTRODUCTION

The Shandon Cytospin® 3 is a cell preparation system which uses centrifugal force to deposit cells onto a microscope slide. The Cytospin 3 maximizes cell presentation for microscopic examination and is distinct from a general purpose centrifuge. Techniques requiring sample concentration or washing steps will require access to a laboratory centrifuge. The Cytospin 3 has operating speed and time parameters of 200-2000 rpm and 1-99 minutes respectively.

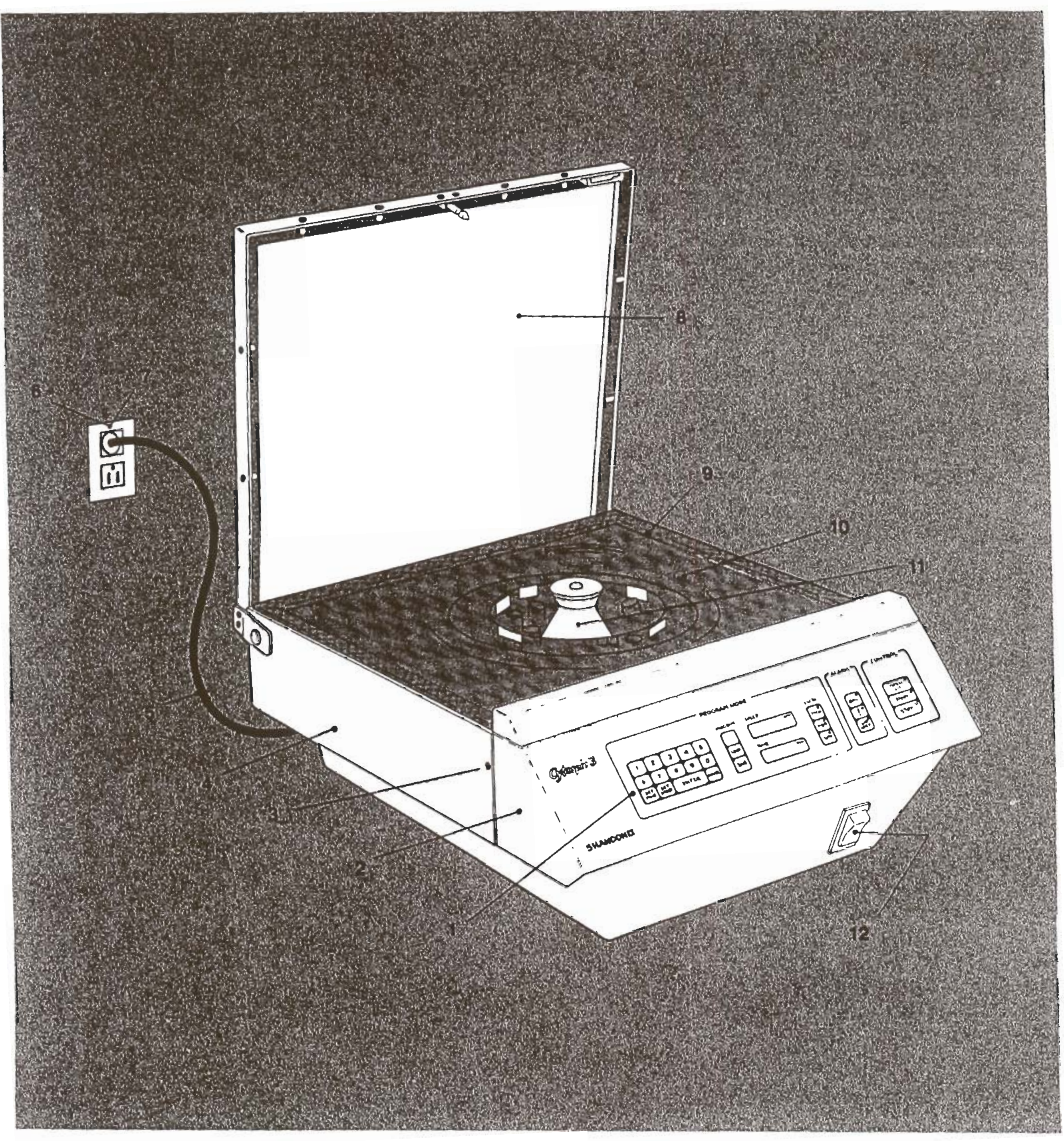
Speed, time and acceleration rate are programmable with the latter available in a choice of either high, medium or low, dependent on sample type used. The instrument also incorporates a nine program selection capability with an indefinite retention time (non-volatile memory).

The Cytospin 3 is supplied with a 12-position sealed head as standard equipment. This head rests on a tapered drive boss and lifts out for routine use.

Samples are centrifuged in reusable plastic sample chambers **or** disposable Cytofunnel sample chambers. Each sample chamber/filter card/glass microscope slide assembly is held in position using a special stainless steel clip (Cytoclip). Up to 12 of these sample chamber assemblies fit into the sealed head. During centrifugation (at 200 rpm) the assemblies tilt from an angled loading position into an upright operating position.



1.2 SET-UP



1 — Wipe-Clean Touch-Control Panel
2 — Nose Cone
3 — Emergency Lidlock Access Cover
4 — Casing
5 — Mains Lead (Electrical Cord)
6 — 200-250V or 100-120V (50/60 Hz) Source with Ground

7 — Earthed Mains Supply or 3-Pin Grounded Outlet
8 — Safety Cover
9 — Silicone Rubber Bowl Liner Seal
10 — Centrifuge Bowl Liner
11 — Sealed Head
12 — Power Switch

1.3 INSTALLING THE CYTOSPIN 3

1 Decide where the Cytospin is to be positioned. Avoid a dusty environment, and ensure that any surface on which the unit is to stand is level and supports the weight of the instrument.

2 Unpack the unit and carefully untie the mains lead (electrical cord).

3 Check the instrument for signs of damage in shipment (broken glass, broken plastic, dents, etc.). If something is missing or damaged notify Shandon or your supplier immediately. Quote the order number(s), inspection number, serial number, date and number of the invoice.

4 Where necessary, fit a suitable plug to the mains lead (electrical cord). Make sure that the earth wire is connected and that the mains supply has an earth (ground) connection.

Instruments supplied to France, West Germany and North America are fitted with plugs during manufacture. UK customers should connect the mains lead to a suitable rated fused plug as follows:

Brown Wire—Live (L or L2) terminal
Blue Wire—Neutral (N or L1) terminal
Green/Yellow Wire—Earth (E or Ground) terminal

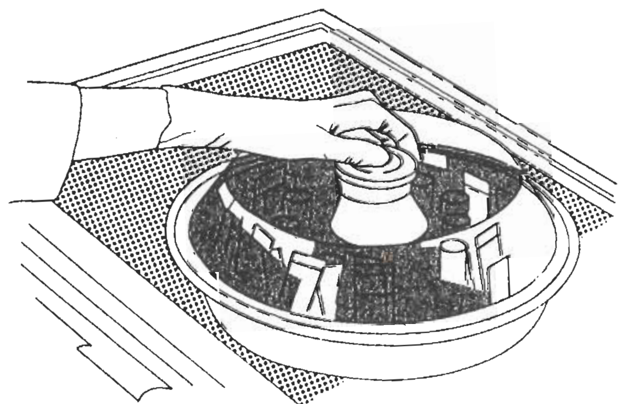
Make sure that the voltage rating on the specification plate at the rear of the Cytospin and on the inspection tag are the same as the mains supply rating.

Switch the instrument on by pressing power switch.

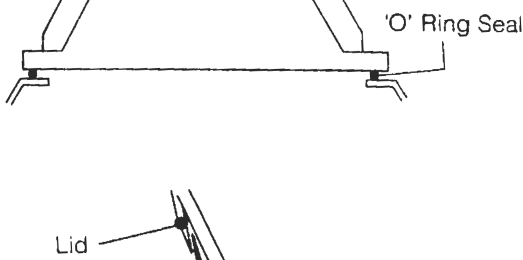
5 Press the 'open lid' button and open the safety cover. In the event of a power failure, the safety cover cannot be opened using the 'open lid' button. The small white cap on the left-hand side of the instrument should be removed. A pencil or rod pushed into the hole and pressed firmly will open the safety cover. Remove the sealed head and set it on the bench beside the instrument. Remove all internal packing.

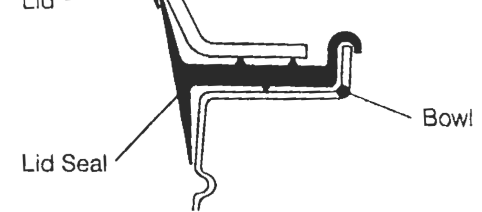
6 Unpack the basic kit and record items on the list below:

a. 1 sealed head with lid and S/S tray with 12 numbered slots. ☐



b. Set of three spare head seals:
1 'O' ring ☐
1 top seal ☐
1 lid seal ☐





c. 12 each Sample Chambers complete with cap. ☐

Filling Port
Location Stud
Outlet Port

Sample Chamber

d. Cytoclip™ slide clips (12 Ea) ☐

Stainless steel Cytoclip™ slide clip

e. One box of 50 Cytofunnel™ disposable sample chambers with caps. ☐

Cytofunnel™ disposable sample chamber with attached filter card

f. One box of white filter cards, 200 each. ☐

g. One box Cytoslide® microscope slides (box of 100). ☐

SHANDON

Cytoslide® microscope slide

7 Record the serial number of your instrument and the date.

Serial Number ____________________________

Date: ________________________________

2. OPERATING THE CYTOSPIN 3

2.1 PRINCIPLES OF OPERATION

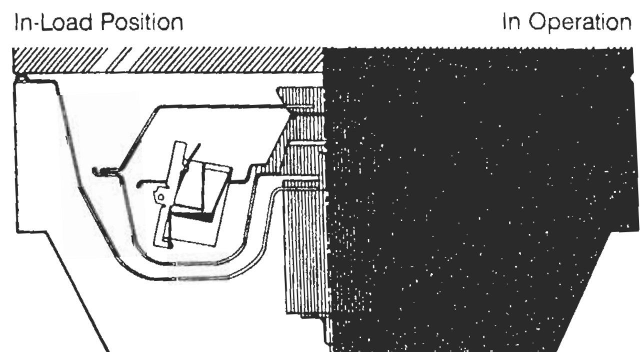
Specimens in cell suspension formed from a wide range of body sites are eligible for Cytospin 3. Spinning forcefully sediments the cells from a suspension onto a microscope slide as the suspension medium is simultaneously absorbed by a blotter.

The result is a monolayer of preserved, displayed cells grouped within an area 6 mm in diameter on a microscope slide. Centrifugal force flattens the cells constructively, offering a more open nucleus and more visually interpretable nuclear detail.

Cytospin 3 uses a unique sample chamber assembly which incorporates a plastic sample chamber, a filter card to act as a blotter, a microscope slide, and a unique stainless steel slide clip. This design ensures that optimum pressure is applied to form an effective seal which controls the rate of absorption.

Up to 12 of these sample chamber assemblies can be run at one time. To prevent cell loss from contact of the cell suspension with the filter card prior to spinning, the sample chambers are inclined in the Cytospin 3 head. This tilting also permits the fluid to be pipetted directly into the sample chambers easily and accurately.

The sealed head with the sample chambers is then lifted onto the Cytospin 3 tapered drive shaft. All sample chamber assemblies remain tilted in the at-rest position so that the specimen cannot trickle forward prematurely onto the filter card.



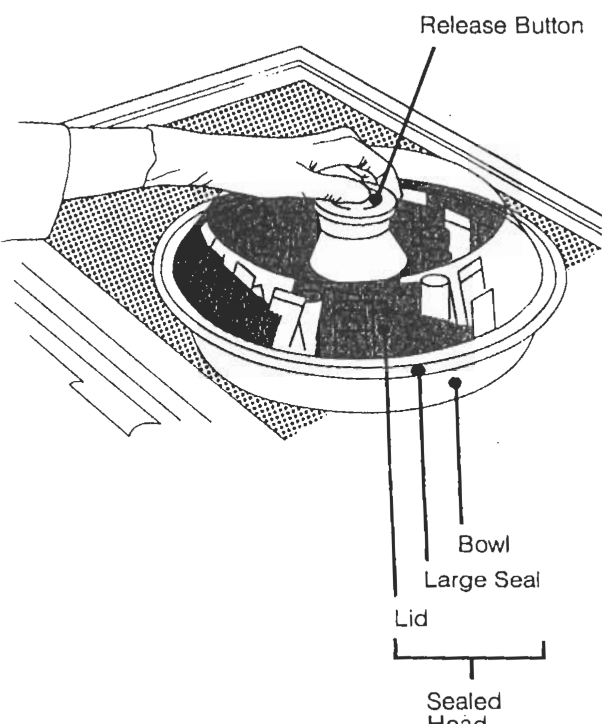
During centrifugation the sample chamber assembly takes a vertical position, allowing the cell suspension to pass through the sample chamber onto the selected area of the microscope slide.

2.2 LEARNING TO OPERATE THE CYTOSPIN 3

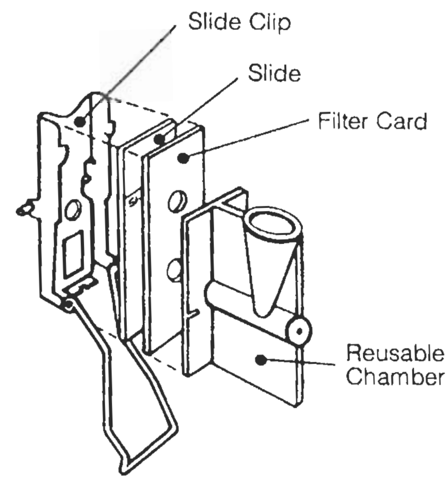
1 Never open the sealed head when it is in the instrument. Remove the sealed head from Cytospin 3.

Release the button in the center of the sealed head by holding the lid with one hand and pulling the release button with the other. Remove the lid and see how the two seals fit into place. The top seal is just under the release button, and the lid seal is the large seal running around the outside of the bowl.

Replace the lid and lock by pushing down on the button in the center of the lid. The sealed head should always be opened and closed while it is **outside** the instrument.



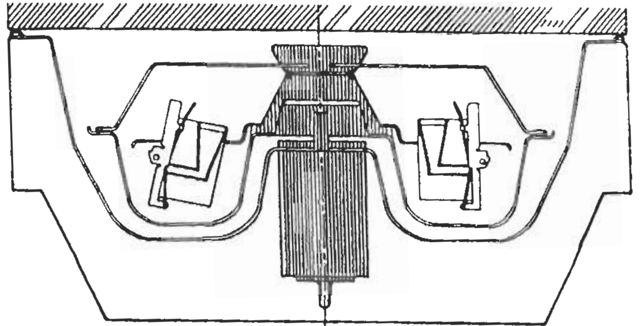
2 With Cytoclip slide clip (Cat. Number 59910052) in open position, fit glass slide, filter card, and reusable sample chamber against the Cytoclip slide clip. Bring spring clip up and secure under the two retaining hooks. Assemble all 12 slide clips with sample chambers, slides and filter cards.



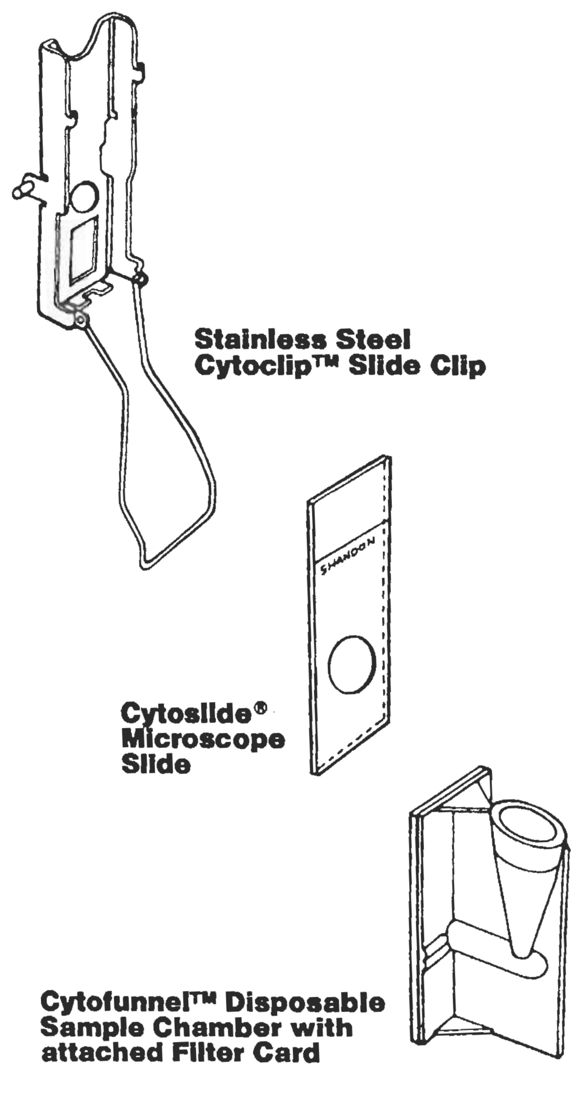
3 Place all 12 chambers and clip assemblies in the slots provided in the bowl. Make sure that each is free to move forward to an upright position and tilt when released. The clip assembly should tilt back toward the center of the head easily of its own accord.

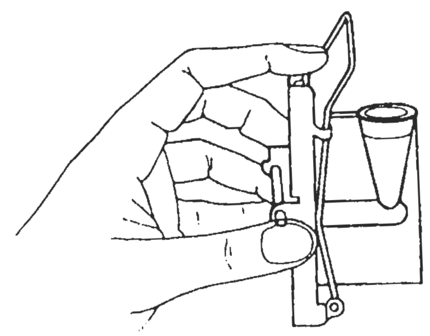
The reason the chamber tilts is so that when the sample is placed into the chamber, it does not run out to the front of the chamber and start to be absorbed by the filter card.

DO NOT PUT FLUID IN THE CHAMBER YET.



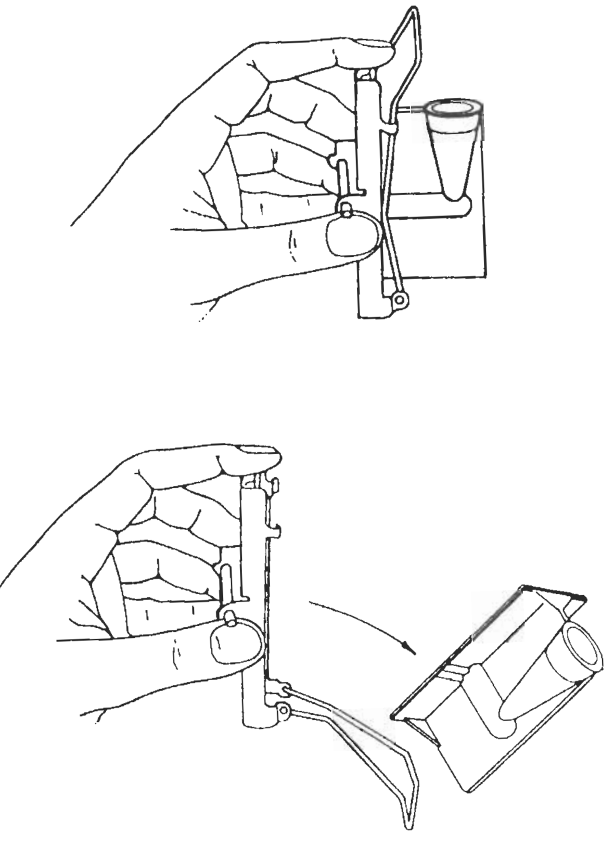
4 Disposable Cytofunnels have permanently attached filter cards. In use, the entire Cytofunnel assembly is coupled with a clean microscope slide and placed in the Cytoclip slide clip.



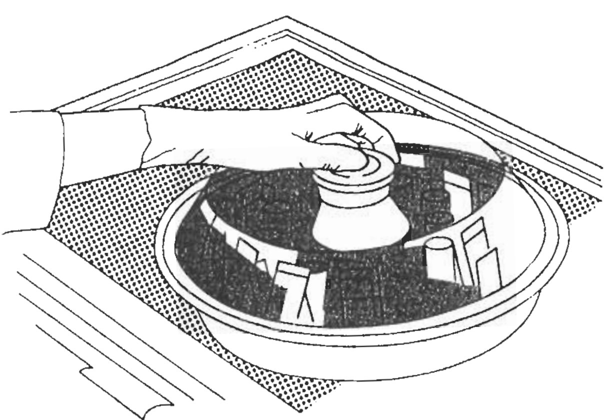


Completed Assembly

Shandon suggest the following method for slide removal following cytocentrifugation. Care must be taken not to brush the specimen along the filter card.



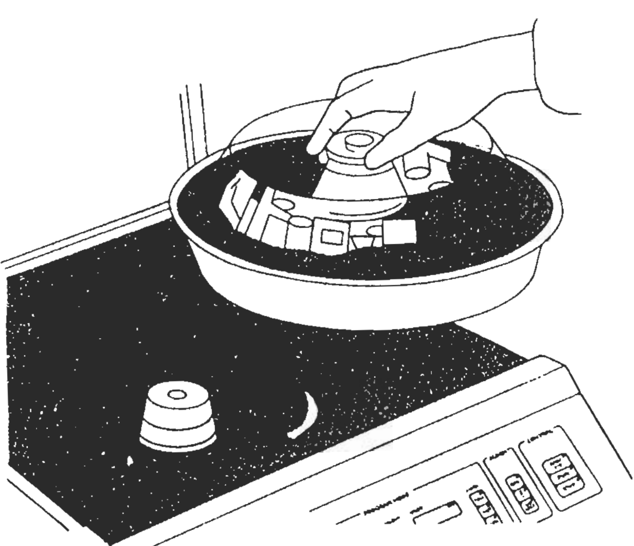
5 With all 12 clip assemblies in place, replace the lid on the sealed head by lifting the center button; now press the center button to lock and seal the head.



Closing the Sealed Head

6 Return the sealed head to the instrument and place it on the center cone. Note there is some silicone grease on the cone. Do not wipe this grease off.

Reapply grease monthly



Fitting the Sealed Head onto the Cytospin 3

2.3 INSTRUMENT CONTROLS, FUNCTIONS AND FACILITIES

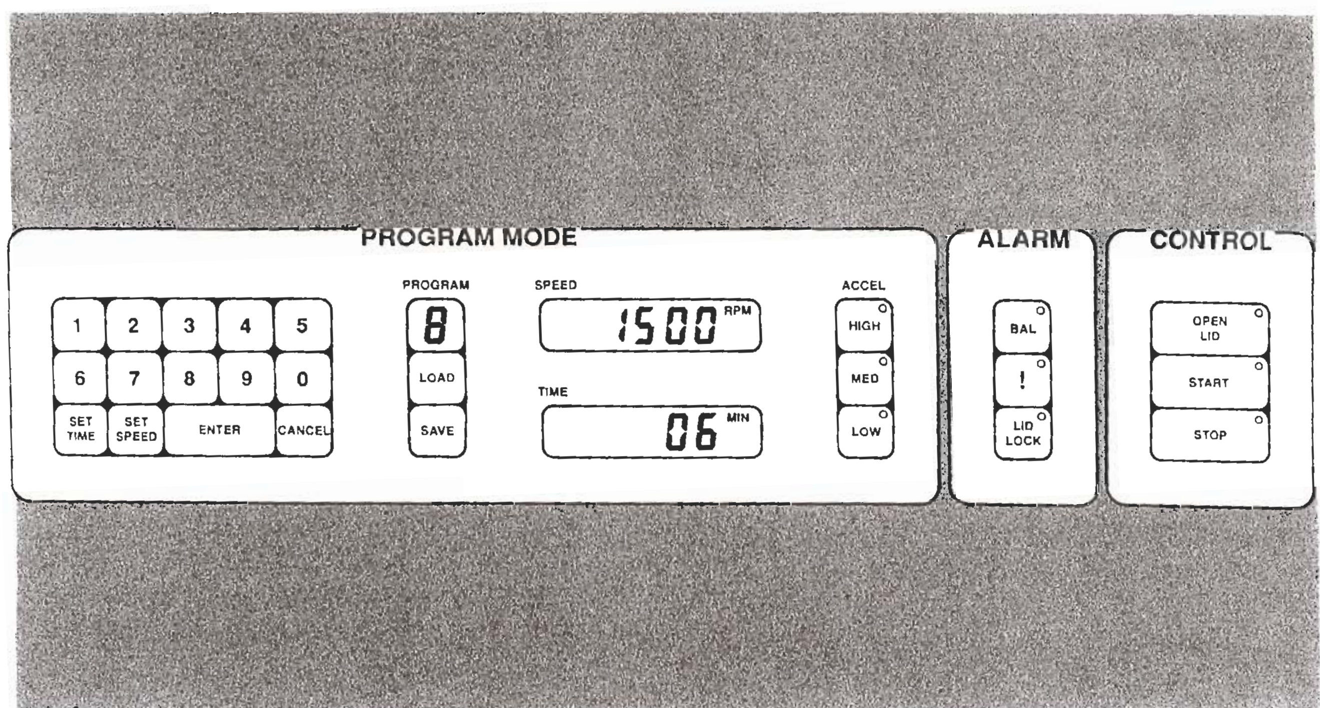
a. Controls and Functions

The Cytospin 3 has a pressure-sensitive control panel. It is only necessary to apply a slight pressure or touch to the center of each area to operate the switch. An audible single tone (signal) is heard for each valid key press and end of cycle. An intermittent tone will be generated for various alarm conditions, should they occur, of which there are 3 indicators (described below). Initialization of the instrument and resetting after run will be prompted by a sequence of tones.

The user interface display has 4 (four) digits of speed (rpm), 2 (two) digits of run time (minutes), and an auxiliary digit showing program number where appropriate. The user interface keys are split into 2 functional groups:

(a) Program Mode—contains function select buttons, 'SET TIME', 'SET SPEED', 'ENTER', 'CANCEL', 'LOAD', 'SAVE', acceleration rates 'HIGH', 'MED', 'LOW', and numerical buttons ranging from 0 to 9.

(b) Control—contains action buttons, 'OPEN LID', 'START', and 'STOP'.



CONTROL	FUNCTION
SET TIME SET TIME	Selects time (minutes) parameter in program memory. Minimum: 1 Minute; Maximum: 99 Minutes.
SET SPEED SET SPEED	Selects speed parameter in program memory. Minimum: 200rpm; Maximum: 2000 rpm
HIGH/MEDIUM/LOW ACCELERATION HIGH MED LOW	Selects acceleration rate. ('ACCEL')
CANCEL CANCEL	When pressed after 'SET TIME' or 'SET SPEED' it cancels time or speed previously entered into memory.
ENTER ENTER	When pressed after 'SET TIME' or 'SET SPEED' and 'TIME' or 'SPEED' value it transfers the time or speed selected into program memory.
START START	Initiates program entered in memory and displays on front panel.
STOP STOP	Stops centrifuge immediately when pressed. The program stored in memory is not affected by using this control.

CONTROL	FUNCTION
SPEED DISPLAY 1500 RPM	Indicates speed programmed or achieved as a 4-digit display. When instrument is first switched on and also when 'SET SPEED' and 'CANCEL' are operated '0' is displayed. When instrument accelerates or decelerates, the display will change as the speed increases or decreases. When the programmed speed is achieved, the speed display remains stable. When the instrument is stopped, the display indicates the actual speed entered into the program memory.
TIME DISPLAY 06 MIN	Indicates time (minutes) programmed or remaining. When instrument is first switched on and at end of program '0' is displayed. When instrument is programmed, but not in operation, the actual time programmed is displayed. When instrument is in operation, the actual time remaining is displayed.
LID LOCK LID LOCK	When illuminated the lid is not securely closed. The Cytospin 3 will not start in this condition. NOTE: The lid lock condition is only checked when the 'START' button is pressed. If there is a serious fault in the electronics causing the instrument to operate over the maximum safe speed, the Cytospin 3 will stop and the '!' display will illuminate.
BALANCE BAL	If the centrifuge head is out of balance, the Cytospin 3 will stop and 'BAL' display will illuminate.
	In addition to the pressure-sensitive control panel, there is an ON/OFF switch fitted at the front of the instrument. The fuse carrier is located at the rear of the instrument.

b. Facilities

Memory

The operator may store and recall up to nine (9) programs. Program retention time will be to all intents and purposes indefinite.
The non-volatile memory holds these units of information:

A. Speed (rpm)

B. Time (minutes)

C. Acceleration Rate (High, Medium or Low)

1. Saving of an entered program is performed through a series of key presses, in the sequence, 'SAVE', 'PROGRAM NUMBER', then 'ENTER'. eg. 'SAVE', '1', 'ENTER'.

2. Alternatively if the operator has already prepared and stored the run information required as program (Prog. Number 3 for example), simply press the keys 'LOAD', '3', 'ENTER', followed by 'START', to run the instrument.

3. Viewing previously entered programs is possible by pressing 'LOAD' and then the numeric keys 1-9. 'SPEED', 'TIME' and Accel. rate being displayed.

Speed/Time Displays

The digital displays give immediate state of the program information and enable the operator to confirm that the correct and stable speed is achieved. Selection of 'SET TIME', 'SET SPEED' will cause the appropriate display to flash until such time as the value and 'ENTER' key has been pressed.

Audible Signal

When the Cytospin 3 has stopped*, an audible end-of-cycle signal sounds for approximately 3 seconds. This tells the operator that:

a. The head may be removed so that the slides may be removed as soon as possible.

b. That a second head can be loaded and the Cytospin 3 restarted immediately, thus maximizing the use of the instrument.

The audible signal is also heard when the 'SET TIME', 'SET SPEED', 'ENTER', 'CANCEL', 'ACCELERATION', 'STOP', and 'START' areas are pressed. This reassures the operator that the pressure sensitive area has been pressed hard enough to activate the switch.

Audible Tones:

Nature of signal	***Indication***
Short tone	Valid key press
Long tone	Invalid key press/ invalid speed entered
Pulsing tone	Alarm condition
3-sec. tone sequence	End of cycle/ head stationary

**Note: The speed display will 'flash' below 50 rpm.*

2.4 PROGRAMMING THE CYTOSPIN 3

This section illustrates how the Cytospin 3 is programmed and how programs may be stored, viewed and run. Please go through each example completely to gain a full understanding of how to effectively use your unit. Ensure that the Cytospin lid is closed before running this unit.

a. Entering and Running a Program:

*Speed Required = 1500 rpm *Time Required = 5 minutes *Acceleration Rate = Low

Key: > < indicates a flashing visual display.

OPERATION	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY	AUDIBLE SIGNAL
1. Switch 'ON'	0	0	HIGH	3 Sec. Audible
2. Press 'SET TIME'	0	>0<	HIGH	valid key tone
3. Press '5'	0	>5<	HIGH	valid key tone
4. Press 'ENTER'	0	5	HIGH	valid key tone
5. Press 'SET SPEED'	>0<	5	HIGH	valid key tone
6. Press '1'	>1<	5	HIGH	valid key tone
7. Press '5'	>15<	5	HIGH	valid key tone
8. Press '0'	>150<	5	HIGH	valid key tone
9. Press '0'	>1500<	5	HIGH	valid key tone
10. Press 'ENTER'	1500	5	HIGH	valid key tone
11. Press 'LOW'	1500	5	LOW	valid key tone

The Cytospin 3 is now programmed. This program may now be initiated **i.e.** run (Operation step 12) **and/or** it may be stored in memory with a designated program number (see b. below).

OPERATION	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY	AUDIBLE SIGNAL	
12. Press 'START'	0	5	LOW	valid key tone	Lid Locked
13. Acceleration Period	0-1500	5	LOW	—	Lid Locked
14. Once Speed Attained	1500	5-0	LOW	—	Lid Locked
15. Deceleration Period	1500-0	0	LOW	—	Lid Locked
16. Head Stops Rotating	0	0	LOW	3 Sec. Audible	Lid Unlocked
17. After 3 Sec. Audible Signal	1500	5	LOW	—	Lid Unlocked

b. Storing (Saving) a Program in Memory:

Program Number 1 designated

OPERATION	PROGRAM DISPLAY	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY
1. Press 'SAVE'	>0<	1500	5	LOW
2. Press Program '1'	>1<	1500	5	LOW
3. Press 'ENTER'	1	1500	5	LOW

Program Number 1 is now entered into the memory. This may be done **either** before **or** after Operation Step 12 above. (**Not** during running of the Cytospin).

c. Running a Program From Memory:

OPERATION	PROGRAM DISPLAY	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY
1. Press 'LOAD'	>0<	—previous program entered—		
2. Press Program '1'	>1<	1500	5	LOW
3. Press 'ENTER'	1	1500	5	LOW
4. Press 'START'	1	0	5	LOW

Program Number 1 has now been recalled from memory and initiated or run.

d. Viewing Previously Entered Program(s)

The operator having stored several programs in memory (up to 9) may wish to later **view** these previously entered programs to inspect speed/time/accel. parameters to act as a reminder in selecting the desired one.

Let us assume that Sections 2.4 a. and b. have been followed, entering 9 different programs in memory, and that the desired parameters are **speed:** 800 rpm, **time:** 8 minutes, **accel:** med, but the actual program number can not be immediately remembered by the operator.

OPERATION	PROGRAM DISPLAY	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY
1. Press 'LOAD'	>0<	0	0	HIGH
2. Press Program '1'	>1<	1500	5	LOW
3. Press Program '2'	>2<	800	8	MED

As you can see, in this example, having sequenced through the above steps, Program Number 2 contains the desired operational parameters (speed/time/accel.) and can now, if desired, be run. Simply follow operational section 2.4 c. 3 through 4.

GUIDANCE NOTES

1. To Repeat a Particular Program

The operator has the choice of either,

(a) Storing a program in memory for future use and subsequently running this program from memory, as detailed in section 2.4 b. and c., **or**

(b) Simply closing the lid following the previous run (with new samples correctly loaded) and pressing 'START'—as detailed in Section 2.4 a. steps 12-17 inclusive.

2. To Alter a Program Mode Parameter (by using the 'CANCEL' button)

(a) Press **'CANCEL'** after 'SET TIME' or 'SET SPEED' to cancel time or speed previously entered into memory. Please note it is essential to then select alternative speed/time parameter(s).

(b) Alternatively pressing 'CANCEL' systematically after having selected the 'ENTER' button for a time/speed parameter, will revert both the speed and time displays to zero.

Pressing the 'CANCEL' button again will cause the speed/time/accel. displays to revert back to the previous program parameters entered.

3. If Program Number 1 Is required (as in Section 2.3 a) but the Lid is not Closed Correctly:

OPERATION	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY	AUDIBLE SIGNAL	LID LOCK	ALARM LED DISPLAY
1. Press 'START'	1500	5	LOW	—	Unlocked & Open	Lid Lock Illuminated
2. Close Lid	1500	5	LOW	—	Unlocked	Lid Lock No Longer Illuminated
3. Press 'START'	0	5	LOW	—	Locked	Lid Lock No Longer Illuminated

From this point follow steps 12-17 in Section 2.4 a.

4. If Program Number 1 is required but the centrifuge head has been loaded incorrectly and is out of balance:

OPERATION	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY	AUDIBLE SIGNAL	LID LOCK	ALARM LED DISPLAY
1. Close Lid	1500	5	LOW	—	Unlocked	—
2. Press 'START'	0	5	LOW	—	Locked	—
3. Acceleration Period 'OUT OF BALANCE' Detected	0	5	LOW	Alarm	Locked	Balance Illuminated
4. Deceleration Period	0-1500	5	LOW	Alarm	Locked	Balance Illuminated
5. Head Stops Rotating	0-1500	0	LOW	Alarm	Locked	Balance Illuminated
6. Switch 'OFF'	—	—	—	—	—	—
7. Switch 'ON'	0	0	LOW	3 sec.	Unlocked	—
8. Reprogram To Display	1500	5	LOW	—	Unlocked	—
9. Correct 'OUT OF BALANCE' Fault	1500	5	LOW	—	Unlocked	—
10. Press 'START'	0	5	LOW	—	Locked	—

11. Follow Section 2.4 a. Step 12 on.

5. Invalid Speeds Lower Than 200 rpm

The Cytospin 3 sample chamber assembly will not 'TILT' into the upright position unless a speed of 200 rpm is achieved. The program memory will reject as INVALID any speed lower than 200 rpm, as illustrated in this example when 190 rpm is entered.

OPERATION	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY	AUDIBLE SIGNAL
1. Press 'SET TIME'	0	>0<	HIGH	valid key tone
2. Press '5'	0	>5<	HIGH	valid key tone
3. Press 'ENTER'	0	5	HIGH	valid key tone
4. Press 'SET SPEED'	>0	5	HIGH	valid key tone
5. Press '1'	>1<	5	HIGH	valid key tone
6. Press '9'	>19<	5	HIGH	valid key tone
7. Press '0'	190	5	HIGH	valid key tone
8. Press 'ENTER'	>0<	5	HIGH	invalid speed

Action to be taken—select and enter a value of 200 rpm or higher.

6. Invalid Speeds Higher Than 2000 rpm.

The Cytospin 3 program memory will reject any speed entered greater than 2000 rpm. As an illustration, examine the following procedures when 2010 rpm is programmed.

OPERATION	SPEED DISPLAY	TIME DISPLAY	ACCEL DISPLAY	AUDIBLE SIGNAL
1. As Number 5 Above Steps 1-4	>0<	5	HIGH	valid key tone
2. Press '2'	>2<	5	HIGH	valid key tone
3. Press '0'	>20<	5	HIGH	valid key tone
4. Press '1'	>201<	5	HIGH	valid key tone
5. Press '0'	>2010<	5	HIGH	valid key tone
6. Press 'ENTER'	0	5	HIGH	invalid speed

Action to be taken—select and enter a value of 2000 rpm **or** lower.

7. Invalid Speed/Time Parameters

The Cytospin 3 will **not** start without a valid speed and time entered and displayed. Similarly a program **cannot** be saved if the speed and time are not valid.

8. ' ! ' Light (Overspeed Indication/Rotor Jam)

The ' ! ' light is illuminated in the event that speed control becomes irregular during a run, due to electrical interference or instrument malfunction.

Similarly should the sealed head assembly fail to start rotating due to mechanical or electrical malfunction, then the ' ! ' light will be illuminated after approximately 3 seconds. An audible alarm signal will sound, and the 'drive' to the sealed head motor will be disconnected.

Turn the power off, and then refer to Section 2.4, No 4, steps 7, 8, 10, 11.

You are now ready to try some actual samples in your Cytospin 3. Please read the technical section, which is next, before you do. You will learn how much sample to put in each chamber and how to handle that specimen before you put it into the Cytospin 3.

Practice removing the sealed head; removing the lid from the sealed head; removing the slide clip assembly and taking the slide and filter card together out of the assembly, before you put a sample in the instrument that you cannot afford to lose.

Refer to the section on Maintenance for instructions on daily cleaning and routine maintenance.

3. TECHNIQUES

3.1 INTRODUCTION

The Cytospin is a special purpose instrument designed to deposit cells onto glass slides. The instrument produces monolayer cell deposition in a defined area of the slide, using centrifugal force. For most cytological specimens, the Cytospin offers significant advantages in specimen retention, preparation standardization, and ease of specimen evaluation.

Cytological specimens may also be deposited onto slides by techniques such as direct smears or by filter techniques. While useful with some specimens, both direct smears and filter techniques have significant disadvantages when compared with Cytospin preparations. Direct smears consistently produce preparations of varying thickness from end to end of the smear. In addition, severe mechanical damage may result to many cells within the preparation. There is also a likelihood of selective cell distribution within the smear. Cells of different size will be deposited in different areas of the smear.

Filter preparations, while excellent for cell retention, are technically demanding and time consuming. In addition, filter preparations rarely yield slides which can be evaluated easily. The cells are seldom in the same plane of focus within the microscope, and it is extremely difficult to obtain well stained cells without also staining the filter. For those filter techniques which dissolve the filter, there is a significant risk of cell loss, in addition to the difficulty and hazards of using a volatile and dangerous solvent.

Cytospin preparations effectively circumvent these difficulties, and consistently produce uniform preparations of cells which are easily stained and evaluated. In addition, the construction of the Cytospin ensures maximum containment of potentially hazardous specimens, thereby reducing risk to laboratory personnel.

Specimens from body fluids and all body sites can be used for Cytospin preparations. The primary requirements are that the specimen be a cell suspension, preferably of single cells, and that the cells are fresh and intact enough to yield diagnostic information. With proper application of the general principles of Cytospin operation, consistent preparations of well preserved cell monolayers should result.

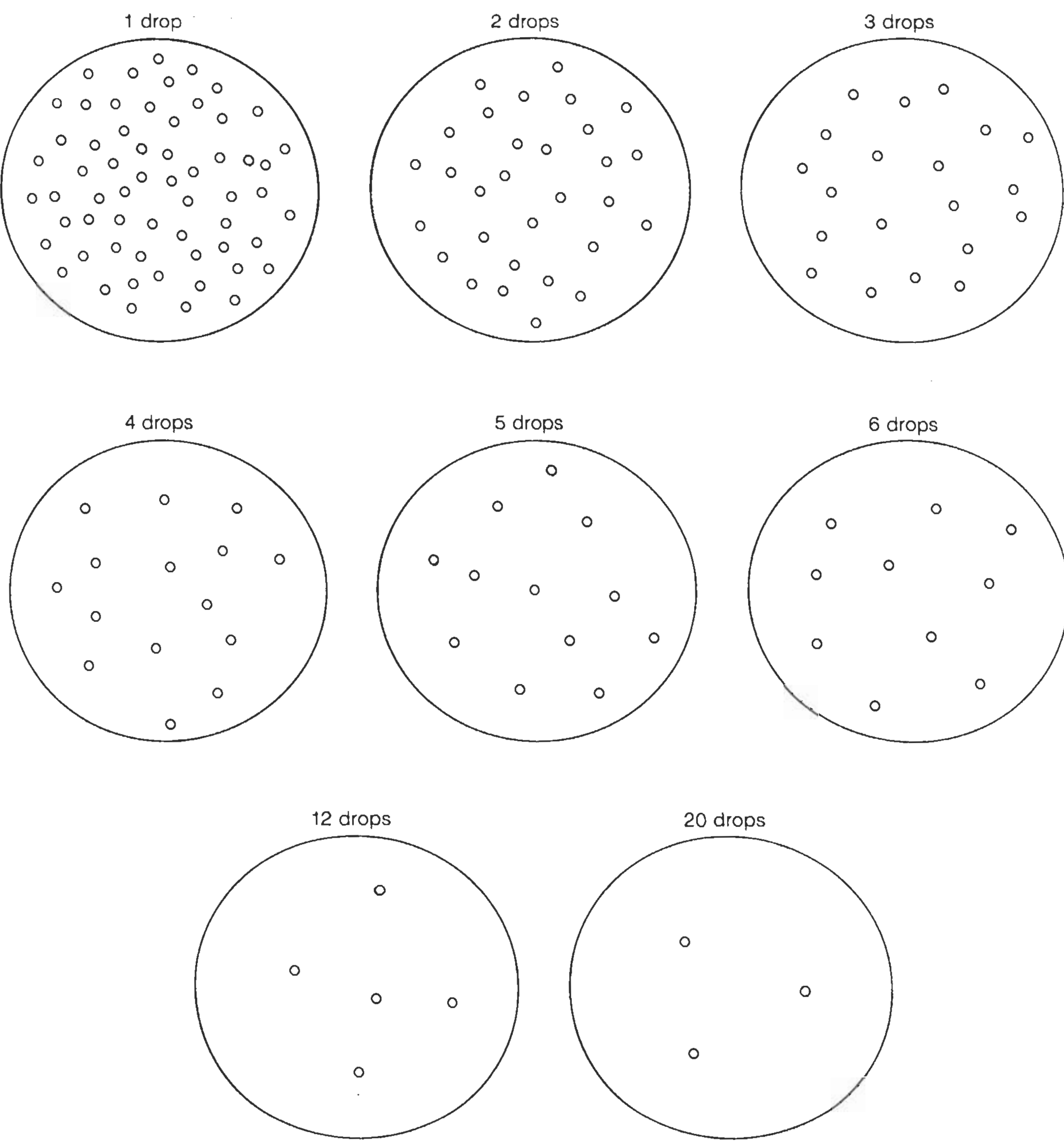
3.2 GENERAL LABORATORY CONSIDERATIONS

The Cytospin is designed to provide maximum protection to the operator by completely containing potentially hazardous specimens. However, the Cytospin cannot protect the operator during the various steps required to process a specimen prior to using the Cytospin. Good laboratory practice requires the use of a biological safety cabinet for all manipulations of cytological specimens. This includes both the loading and unloading steps for the Cytospin. Once the specimen is loaded into the Cytospin sealed head and the lid is sealed in place, the sealed head may then be taken outside the biological safety cabinet for spinning in the Cytospin. After the Cytospin has stopped, the sealed head should be returned to the biological safety cabinet prior to being opened.

Due to the potentially infectious nature of the specimens which may be processed in the Cytospin, the laboratory must establish procedures to ensure that the instrument is routinely disinfected. Suggested methods for cleaning and disinfecting of the Cytospin and accessories will be found in the Cleaning and Maintenance section of this manual.

As with all clinical specimens, it is extremely important to maintain specimen identification. For the Cytospin, this means that the slides onto which a specimen will be deposited must be adequately labelled with the appropriate specimen identification. The method of labelling must take into account the subsequent procedures which will be used. In general, it would be expected that the label may be subjected to fixation steps and staining procedures. Obviously, a paper label would be inappropriate. Pencil identification on frosted-end slides is the most common approach to specimen identification.

The large circles represent cells in one drop of cell suspension, either of unprocessed specimen or preferably of resuspended centrifuged cell concentrate, that have been spread under a 24 x 50 mm cover glass and viewed under a 40X objective. Although drawn smaller than they appear microscopically, the cell and field diameters are proportional to one another at a 50:1 ratio. Simply match the microscopic field with its closest counterpart here and use the number of drops so indicated.



CELLS/40X FIELD AS A GUIDELINE TO THE NUMBER OF DROPS OF CELL SUSPENSION/CYTOSPIN SAMPLE CHAMBER

specimens with tiny cells, cell organelles, or bacteria, may require higher concentrations. The absolute concentration required will be somewhat dependent on the processing methodology employed. As a general rule, the concentration chosen should be such that the cells within the sample have adequate space to spread into a monolayer on the slide surface, with minimal overlap, or piling up of cells. Ideally, the concentration should be high enough that there is not too much space between cells. Having a sufficient concentration of cells speeds up evaluation of the preparation, since little time will be lost in searching for cells to evaluate.

A quick method for approximating the number of cells present in a sample is to place a single drop of the sample on a slide and cover with a 24 x 50 mm coverslip. By lowering the condenser of the microscope, or by closing the microscope condenser diaphragm, the unstained cells can be seen (although detail will not be seen). Using the 10x objective, scan the field and pick an area that appears about average for the entire slide. The cells will most likely not be evenly spread, which is why it is necessay to select an average area. Now switch to the 40x objective. You may also need to open the diaphragm or raise the condenser slightly. Count the cells seen in the field. It is not necessary to count every cell—an approximation will do. Refer to illustration of Cells/40x Field as a Guideline to the Number of Drops of Cell Suspension/Cytospin Sample Chamber, Page 20. This count can be used to estimate the number of drops of the cell suspension required for a Cytospin preparation. To determine the number of cells being used, multiply the number of cells counted by 38. Divide the number of counted cells into 60. The quotient equals the number of drops that should be added to the Cytospin sample chamber, though the total volume should not exceed the 0.5 ml capacity of the chamber. This gives the total number of cells applied to the Cytospin funnel for each drop of suspension used. While this technique for estimation of cell number is an approximation only, it does provide excellent control of Cytospin preparations.

A second method of cell number determination is by manual counting of cells in a hemocytometer. This is a device which defines a precise volume between a special glass slide and a coverslip. By counting the total number of cells within this volume, the cell concentration within the specimen can be accurately determined. While more accurate than simply using a coverslip on a standard slide, the extra precision of this method is not usually required for successful Cytospin preparations.

A third method for determination of cell number is by use of a cell counter of the electronic volume sensing type (Coulter counter). This instrument can provide a precise evaluation of a cell sample. It does require sufficient amount of sample however, which may not always be available. It is common to determine cell number of specimens using this instrumentation in the hematology laboratory. Any specimen obtained from the hematology laboratory may include cell number (or concentration) information.

It is important to recognize that samples which are quite concentrated should be handled carefully. For example, if the specimen is so concentrated that only a single drop may be required, the addition of a second drop will double the cell concentration. It is preferable to work with specimens that are dilute enough that five or six drops are required for the preparation. With such a specimen, the addition of one more drop will not be as likely to result in overlapping cells in the final Cytospin preparation.

While it is common to discuss sample sizes in terms of 'drops', it is important to realize that drop size will be dependent on the type of pipette used to transfer the specimen. As an example, a Falcon 3 ml transfer pipette will dispense approximately 0.5 ml in 15 drops. A six inch glass Pasteur pipette will dispense 0.5 ml in 20 drops. It is advisable to standardize on a single pipette type for all cytological preparations, otherwise 'drops' will be a meaningless measurement.

The laboratory must also ensure that adequate labelling is maintained for all containers or devices to which the specimen is transferred. In use of the Cytospin, this may include one or more intermediate centrifugation steps, conducted in a standard laboratory centrifuge. Each new container to which the specimen is transferred must be appropriately labelled. In addition, the laboratory must ensure containment of the specimen to eliminate potential hazards to laboratory personnel. Since most laboratory centrifuges do not provide aerosol containment during operation, any intermediate centrifugation steps should be conducted in a biological safety cabinet. At the conclusion of specimen preparation, all intermediate containers, pipettes, etc., should be disposed of in an appropriate biohazard container.

3.3 SPECIMEN PREPARATION

a. Initial Examination

Cytological examination always begins with a macroscopic examination of the specimen at the time it is submitted to the laboratory. This is a critical examination, as it provides information which will be used to select a processing protocol. The macroscopic examination is most useful in the hands of an experienced technologist. Prior experience with a particular specimen is invaluable in recognizing whether a given sample is normal or highly suspect, and whether the specimen will be adequate for cytologic examination. However, it is usually impossible to determine if a given sample contains abnormal cells from the macroscopic examination only. A specimen which should normally be clear should not be assumed to be abnormal simply because it is bloody on arrival in the laboratory. Any number of circumstances may produce a different appearance in a specimen during the collection process.

The macroscopic examination cannot be used as a definitive test of the specimen. It does serve to support the eventual diagnostic assessment, but more importantly, it provides the information which will allow the technologist to choose a specimen preparation protocol. A complete macroscopic examination may include:

1. Record of specimen origin—precise anatomical site.
2. Quantity of specimen.
3. Specific gravity (if specimen is **fresh** and unfixed).
4. Odor, if present.
5. Gross characteristics.

Gross characteristics describe the physical appearance of the specimen. Important parameters are the color of the specimen, its viscosity, and whether the specimen is homogeneous or contains solid tissue fragments.

The gross examination will also determine if the specimen is fresh of if it has been fixed prior to delivery to the laboratory. In general, it is preferable if all cytological samples are submitted to the laboratory in the fresh state. However, in many cases, due to transport distances or time constraints, the specimen will be fixed prior to its submission. This must be noted during the gross examination, as fixation may affect several of the parameters recorded during the gross examination. Prior fixation may also constrain the subsequent processing of the specimen. Fixation and its effects will be discussed in a subsequent section of this manual.

b. Determination of Cell Number

Successful operation of the Cytospin requires knowledge of the number of cells present in the sample. While the experienced technologist will achieve reasonable results by estimating cell number, less than optimal preparations sometimes result from such estimates. It is highly recommended that all specimens are examined specifically to determine cell numbers. Visual appearance alone is often confusing, since specimen turbidity may be the result of cell debris, suspended lipids, or other non-cellular materials. In such cases, a direct determination of cell number is necessary to ensure proper Cytospin preparations.

Samples which contain 'average' cells, that is cells with an approximate diameter of ten to twelve microns, produce excellent Cytospin preparations at cell densities of one million cells (1×10^6) per ml. Specimens containing large cells require lower cell concentrations, and

c. Specimen Enrichment

Many cytological specimens arrive in the laboratory as relatively large fluid volumes, many with relatively few cells. Such specimens must obviously be concentrated prior to use of the Cytospin. Such concentration requires the use of a general laboratory centrifuge. The amount of the specimen submitted will determine the size centrifuge tubes which will be necessary. In some cases, the original specimen may need to be split between many tubes. As an example, if a centrifuge is available which can hold 50 ml tubes, and the total amount of specimen is 100 ml or less, then two tubes can concentrate the entire specimen. Should the specimen amount to 150 ml, then four tubes would be required. Remember, centrifugation will also require equal numbers of tubes. By adding more tubes, it is possible to concentrate specimens which are quite large in volume. To sediment the cells, the centrifuge should be spun at 2000 to 3000 rpm for 10 to 20 minutes. Avoid spinning at excessive speeds. This will only damage cells, and pack them into such tight buttons they will be difficult to process further. Centrifuges with swinging buckets will generally require slightly higher speeds than angle-head centrifuges.

After conventional centrifugation, any cells present in the sample would appear as a packed button in the bottom of the tube. The clear supernatant above the cell button should be carefully aspirated or poured off, leaving a volume of fluid approximately equal to the volume of the packed cell button. The fluid that is aspirated or poured off may be discarded, using any common technique to render it harmless (sterilization, fixation, etc.). This fluid should only be discarded after it is determined that the cells within have indeed been retained.

The packed cell button in the bottom of the centrifuge tube should be thoroughly mixed with the residual fluid which was not removed. This is done either by use of a vortex mixer, or by gentle agitation of the tube. The result is a concentrated cell suspension, suitable for cell number determination, and subsequent preparation with the Cytospin.

d. Specimen Dilution

Cytology specimens often are submitted to the laboratory with a cell concentration that is too high for Cytospin preparations until diluted. Such specimens are common from bone marrow, lymphoid aspirates, and many fine needle aspirates. These concentrated specimens should first be evaluated for cell number, using any of the previously described techniques. The specimen should then be diluted to an appropriate cell concentration by addition of a balanced electrolyte solution. It is important to use a fluid that has a proper osmolarity, in order not to introduce structural changes in the cell sample. Simple solutions of sodium chloride (0.9% saline) are unsuitable as diluents—they produce rapid changes in nuclear chromatin and interfere with subsequent cytological examination.

Suitable diluents are those commonly used in hematology laboratories, such as Polysal, Polyonic R-148, Plasma-Lyte, Isoton, Tisu-u-Sol or Normosol. Many of the solutions commonly used in tissue culture laboratories are also suitable diluents, such as Gey's balanced salt solution, Earle's balanced salt solution, or Hank's balanced salt solution. In many cases, if the cell suspension submitted to the laboratory has been collected in one of these diluents, or has undergone significant processing in such salt solutions, it may be advisable to add some protein to the diluent. Either human serum or a solution of bovine serum albumin may be used. The usual concentration of these solutions is 1 to 30 percent. The protein solutions of very high concentration are usually used by dropwise addition to the sample just prior to or during loading of the Cytospin funnels.

e. Loading the Cytospin Sample Chambers

The sample chambers hold a maximum of 0.5 ml of specimen and should hold no less than 0.1 ml. It is mandatory that no more than 0.5 ml of sample is placed in each sample chamber. Additional sample would simply be thrown to the top of the chamber during Cytospin operation, and could not be deposited on the slide. It is recommended to load the sample chambers after they have been assembled and inserted into the sealed head. The design of the chamber assemblies and the sealed head ensures that the sample chambers tilt in such a manner that the specimen will not contact the slide or the filter card prior to

starting the Cytospin. The specimen must **never** contact the slide or filter before the Cytospin is started. The operator must be careful during loading not to forcibly inject the sample into the sample chamber. The sample should be eased into the chamber slowly, allowing ample opportunity for air to be displaced by the sample. Cytofunnel™ disposable sample chambers are available for added safety and convenience.

For concentrated cell suspensions which require only one or two drops of sample to obtain the correct cell concentration, it may be sometimes necessary to add additional diluent to bring the total volume in the sample chamber up to 0.5 ml. This addition can be done in the chamber as the samples are loaded. However, this requires care to avoid forcing sample into the slide/filter area, and it is recommended that 'thick' specimens be diluted prior to being loaded into the Cytospin.

During loading of the sample chamber, the sample should be deposited directly in the bottom of the sample chamber. Avoid dripping the sample down the side of the sample chamber. Should sample be deposited on the walls of the chamber, rinse down with a drop of diluent. The object is to ensure that all of the sample is in the bottom of the sample chamber.

f. Selecting Time and Speed for the Cytospin

The speed of operation of the Cytospin is dependent on the size of the cells or particles to be deposited on the slides. In general, average cellular specimens will require a speed of approximately 1000 rpm. Very large cells and fragile cells may require a slower speed, such as 500 to 800 rpm. Specimens consisting of tiny objects such as bacteria may require much higher speeds, approaching 2000 rpm.

Time of Cytospin operation is also related to specimen type and to subsequent preparative steps. For most cytological preparations, it is desirable to avoid any possibility of air drying of the specimen. Therefore the time used for Cytospin operation is kept as short as possible, such as 3 to 4 minutes. For hematology and microbiology specimens which often are air dried prior to further processing, a longer time is used, often approaching ten minutes.

An appropriate Cytospin time will ensure fluid absorption. The cells on the slide should have a thin layer of fluid on their surfaces. Occasional specimens may be too thick to completely absorb in the filter paper during a normal time and speed setting. Such specimens may require special processing. An example is joint aspirations which contain hyaluronic acid, giving them a thick consistency. This can be reduced by adding a small amount of the enzyme hyaluronidase to the sample prior to operation of the cytocentrifuge.

g. Unloading the Sample Chambers

After the Cytospin stops, the specimens should be removed as quickly as possible. The lid of the Cytospin is opened, and the sealed rotor is removed and taken to the biological safety cabinet. The lid is opened, and the individual sample chamber assemblies are removed from the rotor. For cytology specimens, the assemblies are laid flat on towelling, and the clips released. The chamber assembly is lifted straight up on occasions where liquid remains, allowing any residual fluid to flood the slide. If there is a considerable amount of residual fluid, wait until some of it evaporates. However, do not allow the specimen to dry. Just before drying begins, place the slide into fixative, or spray with Cell-Fixx™.

For specimens which do not contain excess fluid, quickly remove the sample chamber, remove the slide, and immediately place into fixative. (This is best achieved by easing the slide into the fixative, so as not to disturb the deposited cells.)

Specimens intended for air drying techniques, such as hematology specimens and some microbiology specimens, are handled somewhat differently. In these specimens, it is undesirable to have residual fluid flood the slide. The chamber assemblies are stood upright on the towelling prior to unclamping. Then the clips are released and the chamber pulled away from the slide. Residual fluid is retained in the sample chamber, and does not flood over the deposited cells. The slides resulting from this procedure are then completely air dried prior to further processing.

Occasionally samples will be received that have been fixed in some other fixative such as formalin. These will have a different nuclear and cytoplasmic appearance if processed without exposure to alcohol. Such specimens can be concentrated, the formalin poured off, and then resuspended in Saccomanno-type fixative. The result will be a specimen that is reasonably similar to those fixed in the alcohol fixative alone.

Fixation makes cells more rigid, and less able to spread when placed in the Cytospin. Specimens that have been fixed prior to depositing on slides will require slightly higher speeds and longer times to achieve the same degree of spreading as seen in unfixed specimens. Occasionally the laboratory will be asked to prepare specimens that have been held in fixative solutions for extended times. These specimens may be so rigid that it is difficult to get them to flatten on the slide. The addition of a small amount of glycerol to the specimen, allowing some 'soak' time, followed by use of the Cytospin, will usually result in a reasonable preparation.

Many laboratories prefer to fix all specimens during preparation. The usual protocol is to concentrate the specimen, then resuspend in an equal volume of Saccomanno-type fixative. For samples that must be diluted, the diluent can be fixative. These fixation steps are generally done just before adding the sample to the Cytospin sample chamber assemblies.

Whether the specimen has been fixed before deposition on slides or not, immediately after removing the slides from the Cytospin, they should be immersed in 95% alcohol to complete fixation and dehydration. Since the cells will still be wet, and will not have become totally bound to the glass slide, use care in this transfer. (Ease the slide into the fixative in the container.) Many complaints of poor cell capture can be traced to lack of care in this step of the procedure.

3.4 SPECIAL CONSIDERATIONS

a. Cell Adhesion

Successful application of the Cytospin requires that cells adhere to the glass slides. For many routine applications, it is sufficient to use clean slides. Slides may be cleaned using alcohol. The increasing use of long staining techniques such as immunostaining may require additional steps to ensure adhesion of samples. Slides may be coated with Poly-L-Lysine (Sigma) or with aminosilane (Sigma). Gelatin coated slides may also be used. The use of any of these adhesion methods will increase cell retention and reduce the incidence of 'floaters' in the subsequent staining baths.

b. Cytology

The majority of the procedures previously discussed apply to cytological specimens. However, there are a number of specific specimen types which require special processing. Often, the cytology specimen will contain clots, fibrin webs, or tissue fragments. These will all interfere with the Cytospin preparations. Small floating clots and fragments should be removed with forceps. These may be saved for cell block procedures. Fibrin webs are generally too friable to be removed intact. These are most easily removed by twirling a glass or wooden rod in the specimen. The fibrin web is wound onto the rod, and in the process, many of the trapped cells will float free. After winding on the fibrin, it is pressed gently against the side of the container to squeeze out as much of the trapped fluid and cells as possible. As with all cell manipulation techniques, it is important to be gentle to avoid excessive cell damage.

Cytological samples may contain considerable quantities of mucus. This produces a very thick mass that is difficult to dilute or concentrate, and becomes a rubbery mass on fixation. Such specimens should be processed prior to fixation. A common way to break up mucus is to mix with an equal part of Saccomanno-type fixative and immediately blend in a small blender. Three to five seconds is usually sufficient. The blending procedure should be done in a biological safety cabinet. After blending, the sample should be homogeneous and non-stringy, and can be deposited using the Cytospin. Certain chemicals also react with mucus to produce liquefaction, such as acetylcysteine (Boccato, 1981). A commercial product of this type is Mucolexx, which contains not only a mucolytic agent but Saccomanno-type fixative as well.

h. Evaluation of Specimen to Assess Technique

After staining, the specimens can be evaluated to assess the preparative technique. The ideal result is a monolayer of cells with minimal overlap, yet sufficiently concentrated that one does not have to search for cells in the preparation. The cells should display excellent morphology. There should be no evidence of stretching, or tearing of the periphery of cells. Such artefacts indicate excessive speeds or times of cytocentrifugation. In excellent preparations, there will be flattened nuclei with distinct chromatin patterns. Some cell types, such as columnar epithelial cells should retain their typical columnar morphology. Distortion of their columnar shape indicates excessive speed or time of cytocentrifugation.

Occasionally one will see a specimen that has a pattern of cell deposition around the periphery of the deposition spot, with a loss of cell in the center. This effect is due to an excess amount of residual fluid in the center of the cell deposition area when the specimen is fixed. Because the cells in the center of the area are quite wet, they wash off the slide as it is immersed into the fixative. The solution to this problem is to allow a longer time for the slide to dry prior to fixation, and to be exceptionally gentle during immersion of the slide into the fixative.

j. Fixation

Fixation is used to preserve cell samples, to render them more easily stained, and to produce characteristic patterns of cell structure which are used to distinguish cell types. Cells continue their natural living processes after being removed from body sites. Since they no longer have their normal blood supply and other supporting environment, they will begin to degenerate as they run out of required nutrients and gases, and begin to build up waste products. As these events continue, the cell activates internal repair mechanisms that eventually result in the cell digesting itself. This is called autolysis. The rate at which autolysis progresses is different for different cell types, but does mean that samples should be processed as quickly as possible. Autolysis can be slowed significantly by refrigeration, and samples may be held for some period of time at refrigerator temperature. Where practical however, specimens should be fixed or processed as soon as possible.

Fixatives are chemical agents that both kill the cell and stabilize its structure. The 'killing' also inactivates many of the enzyme systems of the cell, particularly those associated with autolysis. Fixatives therefore also function as preservatives, and well fixed cytological samples essentially last indefinitely. Many specific chemicals can be used as fixatives, and each has specific properties that are desirable for certain types of study. These fixatives include those that produce chemical cross-links within the tissues, such as formalin, and those that precipitate cellular components such as the alcohols. By far the most common fixative used in cytological studies is alcohol. Alcohol produces distinct nuclear chromatin patterns, and also serves to remove water from cells. Cell-Fixx spray fixative is an example of an alcohol-based cytological fixative which also contains †Carbowax.

A disadvantage of alcohol fixatives is that they evaporate quickly, and therefore there is always the risk of permitting specimens to dry out. To avoid this, many laboratories use Saccomanno fixative, which is a mixture of 70% ethyl alcohol and 2% Carbowax (polyethylene glycol). The Carbowax in this mixture forms a coating over the specimen, helping protect from the effects of drying. The Carbowax is soluble in water, and so is dissolved in subsequent staining steps. Commercial versions of this fixative are available (Shandon Cytospin Collection Fluid).

When specimens must be transported over considerable distances, or when they cannot be processed immediately, it is advisable to fix with alcohol or with Saccomanno-type fixative. This is done by adding an equal volume of fixative to the sample. If the specimen is large in volume, the sample should be centrifuged to concentrate the cells, and then fixed. Immediately after adding the fixative to a sample, the sample should be vigorously agitated to suspend the sample within the fixative.

†Carbowax is a registered trade name of Union Carbide.

3.5 USE OF THE CYTOSPIN FOR HEMATOLOGY AND OTHER CLINICAL MICROSCOPY SPECIMENS

a. Introduction

This section of the manual is designed to provide general guidelines for the use of the Cytospin in hematology and clinical microscopy.

b. Uses and Applications

The Cytospin has a wide range of applications in clinical microscopy, some of which are shown below:

USES	CLINICAL APPLICATIONS
1. Romanowsky-stained cytology of CSF and other body fluids.	Evaluation of possible infection or presence of malignancy.
2. Gram stain and other special stains of CSF and body fluids.	Detection of infectious agents: characterization of malignant cells.
3. Slide preparation from ficoll-hypaque cell isolates	Provides slides for morphology, cytochemical staining (e.g. myeloperoxidase, non-specific esterase), and immunocytochemical or immunofluorescent assays (e.g. TdT). Used to characterize leukemias and lymphomas.
4. Cell surface and cytoplasmic marker studies using monoclonal antibodies.	Classification of leukemias and lymphomas.
5. Urine eosinophils.	Evaluation of drug-induced nephritis, allergic cystitis, and renal transplant rejection.
6. Urine hemosiderin.	Confirmation of severe intravascular hemolysis and chronic iron overload.

c. Methodology Guidelines

When first setting up the Cytospin for use, it is helpful to establish a standard procedure, i.e. amount of specimen/slide, rpm and minutes centrifuged, and maximum number of white blood cells/µl and red blood cells/µl, above which dilutions would be necessary.

1 Determine the red cell and the white cell count of the sample according to established methods.

2 Using a 'standard' amount of specimen, (e.g. 5 drops or approximately 0.25 ml) make serial dilutions of a highly cellular specimen to determine the maximum number of white blood cells and red blood cells that can be present in a specimen before dilutions are necessary. Some tests may require more cellular slides than others.

3 Experiment with a range of rpm to see which gives the most desirable morphology for the procedure involved. Establish the minumum number of minutes required to spin the entire standard amount of specimen onto the slide. Experiment with the 'high' and 'low' acceleration settings to see if there is any difference in morphology of the cells.

4 The following is an example of a standard procedure for preparing slides for a white cell differential with Wright stain:

Use 5 drops of specimen/Cytospin chamber. Dilute sample to obtain a white cell count less than 500/µl and a red cell count less than 5,000/µl. Centrifuge at 700 rpm for 5 minutes.

Dilution Chart for WBC Dilution:

WBC COUNT	DILUTION
0-500	NONE
501-1000	1/2
1000-1500	1/3
1501-2000	1/4
2001-2500	1/5
2501-3000	1/6

Bloody or serosanguineous specimens may contain so many erythrocytes that examination of cytological preparations is difficult, and when the specimen is diluted sufficiently to obtain monolayer preparations, the cells of interest are difficult to locate. Red blood cells may be removed by gradient centrifugation or by various lysing procedures. Lysing techniques are commonly used for leukocyte counting on cell counters which employ sensing orifices. A number of commercial lysing reagents are available and provide complete destruction of erythrocytes. Lysing reagents may also damage some cells of cytological interest, and care must be used in their use. The large amounts of hemoglobin released from the red cells may interfere with subsequent staining. It can be removed by several washing steps using a conventional centrifuge.

Gradient centrifugation is based on a density difference between red cells and other cells within a specimen. Commercial gradients are available, and are based on mixtures of Ficoll and Hypaque. The sample is layered onto the gradient in a centrifuge tube, and then the tube is centrifuged. The red cells will migrate through the gradient, and will also be hemolyzed. The remaining cells will stay on top of the gradient, and can be removed for subsequent processing.

c. Hematology

The primary difference between cytological and hematological preparations is the routine use of air dried preparations in hematology. In most cases, hematology will also have available a specific cell count derived from an electronic volume sensing instrument. This permits a defined dilution and allows precise control of cell number on the final Cytospin preparation.

Hematological samples are routinely diluted to obtain samples of the correct cell concentration. The diluent used is commonly one of the balanced salt solutions. It is advisable to add a small quantity of serum albumin (preferably bovine, for safety) to the sample to increase adhesion of the cells to the slide, and to avoid the deleterious effects of the high salt concentration as the diluent evaporates during the drying of the slide.

Since hematology specimens are to be air dried, they should not be flooded with any residual liquid after use of the Cytospin. For hematology, after the sample chambers are removed from the Cytospin sealed head they are stood upright. This drains any residual fluid back into the sample container. The clips are then removed, and the slide separated from the sample container and filter. The slide is then air dried. As with all air dried preparations, the more rapidly drying occurs, the better. Drying may be accelerated by gentle heating of the slides.

d. Urine Specimens

Specimens derived from urine are typically high volume fluids with few cells or particles. These specimens must be concentrated by conventional centrifugation prior to cytologic examination. Often urine specimens contain particles which are not cellular but are precipitated phosphate salts. It is often suggested that one can dissolve these particles by acidifying the specimen with a few drops of acetic acid. While this will cause the salts to dissolve, acetic acid is also a classic fixative. It will cause chromatin condensation in cell nuclei, and will also produce significant cell swelling. Acetic acid is a component of Carnoy's fixative. If acetic acid is used, its effects must be accounted for in subsequent evaluation of the specimen.

e. Microbiology

Many microbiology samples are quite similar to cytological samples. They will contain cells, and the microbiologist is interested in the association of bacteria or viruses with the cells. The Cytospin can also be used to directly deposit samples of bacteria onto slides. The advantage of this use is that the deposited bacteria are generally more concentrated than after simple smearing, and they are all located in a defined area of the slide. Due to the small size of bacteria, the Cytospin is generally operated at speeds of 2000 rpm for five to ten minutes.

Many of the techniques used for localization of viruses or bacteria require the use of fixatives other than alcohol based ones. In general, techniques using immunostaining or nucleic acid probes will specify the use of aldehyde fixation, such as formaldehyde (paraformaldehyde) or glutaraldehyde. These do not affect the operation of the Cytospin, since they are applied to the slide after the cells, bacteria, or viruses are deposited on the slide. It is important to use a slide adhesive, preferably Poly-L-Lysine or aminosilane for these preparations.

If the red cell count which results from the above WBC dilution is greater than 5,000/μl, calculate the further dilution necessary to bring the red cell count below 5,000/μl. Use this dilution regardless of the fact that the white cell count may be very low.

5 Assemble the sample chamber assembly as per the instructions in the Operating Section. Be sure to align the outlet hole of the sample chamber exactly inside of the hole in the filter card. The edge of the sample chamber must not touch the sample card or the cell yield will be greatly reduced.

6 Always place the clip assembly into the Cytospin head before adding anything to the sample chamber. Check that the assembly pivots freely in the metal support plate.

7 Add one drop of 30% albumin to the bottom of the sample chamber, then add the standard amount of diluted fluid. Cap the sample chambers. The albumin helps to preserve the cells and keep them intact as they are deposited onto the slide. (Note: albumin may quench fluorescence and should not be used when immunofluorescent assays are to be performed).

8 Lock the lid down on the base, transfer the sealed head to the Cytospin, and close and lock the lid. Never snap the lid of the Cytospin head down onto the bowl while the assembly is sitting on the drive shaft, as this may damage the shaft.

9 Program the Cytospin for the standard number of rpm and minutes and start the unit. Fragile cells may require a lower rpm setting and low acceleration to maintain morphology.

10 When the alarm has ended, remove the sealed head and transfer it to a hood before opening.

11 Check the sample chambers to see if all of the specimen has spun onto the slide. Make sure that any remaining specimen in the chamber does not flow onto the slide as it is removed from the clip. Should this occur, the cells will not remain spread out on the slide and may therefore overstain.

d. Suggestions for Optimal Cytospin Technique

1 Use one drop of 30% albumin at the bottom of the Cytospin chamber.

2 Do not make a push smear, even when the cell counts are high. Large malignant cells are difficult to identify on push smears because they may aggregate at the feather edge or stain darkly in the thick portion of the smear.

3 Centre the outlet port of the sample chamber exactly inside of the hole in the filter card.

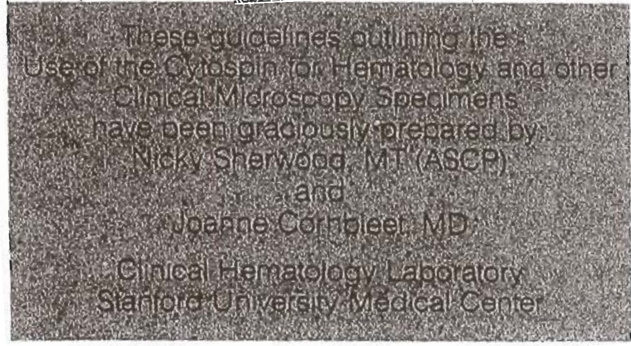
4 Do not let unspun specimen wet the slide when the chamber is disassembled.

5 If fibrin strands or other contaminant materials are present, they may clog the filter card and prevent absorption of the specimen. Better slides may sometimes be obtained if an aliquot of the specimen is first diluted in saline, centrifuged, and then the cells are resuspended in saline to the original volume.

6 If synovial fluid is extremely viscous, a small amount of hyaluronidase may be added to liquefy the sample before processing.

7 If a fluid is clotted, Cytospin slides may be prepared from a suspension of the clotted material as well as from the unclotted fluid to increase the possibility of detecting malignant cells.

8 Avoid bacterial contamination of the albumin or saline diluent. Report the presence of bacteria on the slides only when they are also present in neutrophils. Make sure that slides prepared with **reagent only** are free of bacteria.


These guidelines outlining the
Use of the Cytospin for Hematology and other
Clinical Microscopy Specimens
have been graciously prepared by
Nicky Sherwood, MT (ASCP)
and
Joanne Cornbleet, MD
Clinical Hematology Laboratory
Stanford University Medical Center

4. TROUBLE-SHOOTING GUIDE

4.1 QUALITATIVELY ABERRANT CYTOCENTRIFUGATION RESULTS

PROBLEM	CAUSE	SOLUTION
	Before Cytocentrifugation	
Cells preserved poorly	Preserved poorly in vivo	Request repeat specimen
	Lengthy delays between collection and preparation	Minimize delays, (e.g. less than 4 hours); refrigerate, if longer delays
	Cells suspended in normal saline	Use balanced electrolyte solution
Cells small in diameter, optically dense	Cells collected in high proportion of strong alcohol	Collect unfixed fresh specimens, or mix with equal volume of 50% ethanol
	Alcohol added to sample chamber begins to rise through the cell suspension, and upon mixing with it, causes cellular shrinkage	Add less alcohol; add alcohol carefully
RBCs hemolysed, ghosts remain	Alcohol has mixed with cell suspension	Carefully add alcohol to sample chamber
	During Cytocentrifugation	
Cells air-dried	Cell suspension medium absorbed completely by blotter	Fill cylindrical sample chamber before cytocentrifugation; use Cytospin® Collection Fluid; reduce cytocentrifugation speed and/ or time
Cells air-dried around periphery of collection area	Cell suspension medium absorbed almost completely by blotter	Increase specimen volume up to 0.5 ml; use Cytospin Collection Fluid; reduce cytocentrifugation speed and time
	After Cytocentrifugation	
Cells air-dried	Film of liquid over cells allowed to evaporate during brief interval between unloading and immersion in alcohol	Move quickly to avoid evaporation of protective thin film from over cells
Cells air-dried around periphery of collection area	Film of liquid is thinnest at its edges and so evaporates before the thick central area evaporates	Immerse cells in alcohol before air-drying progresses onto periphery of cell collection area
Completely disrupted air-dried pale cells, resemble 'basket cells' of hematology	Fragile cells air-dried and exaggerated by centrifugal force	Request repeat specimen, do not allow to air-dry

4.2 QUANTITATIVELY ABERRANT CYTOCENTRIFUGATION RESULTS

a. Number of Cells

PROBLEM	CAUSE	SOLUTION
	Before Cytocentrifugation	
No cells	Exit port blocked by blotter	Seat blotter to foot of slide clip
Abnormal cells in specimen, but not on cytocentrifuged preparations	Abnormal cells are usually larger and heavier than normal cells and so sediment to very bottom of cell concentrate. May be missed if not resuspended completely following conventional centrifugation	Apply centrifuge tube with cell concentrate and several ml of balanced electrolyte solution to vortex mixer to completely resuspend the cells
Too few cells	Too few cells in raw specimen	Enrich first by conventional centrifugation; resuspended cells in 1-2 ml balanced electrolyte solution Combine contents of multiple centrifuge tubes of same specimen when possible Microscopically examine drop of resuspended cell concentrate; base sample size on cell count Request repeat specimen; suggest to clinician ways to increase cellular harvest
	Too few cells added to sample chamber	Base sample size on cell count of drop of resuspended cell concentrate
	Sparsely populated specimen may have filled the cylindrical and conical portions of the sample chamber	Enrich specimen as described above Add specimen only to cylinder of sample chamber
	Partially filled cone raised level of specimen in cylinder to blotter where cells can be absorbed	Do not allow distal boundary of specimen to touch blotter before cytocentrifugation
	Cells crowded by precipitated hyaluronic acid in joint fluid	Dissolve hyaluronic acid precipitate with pinch of hyaluronidase
	Cells crowded out by precipitated phosphate salts in urine	Mix in several drops or more of glacial acetic acid to lower the pH and redissolve the alkaline pH-dependent precipitated phosphate salts
	Cells crowded out by erythrocytes	Saponinize specimen
	Slide between exit port and blotter	Load in correct sequence: sample chamber-blotter-slide
Too many cells	Too much densely populated cell suspension added to sample chamber	Microscopically examine drop of resuspended cell concentrate; dilute up to 10X if necessary; base sample size on cell count or derive it from hematological counting chamber Do not rely on visual estimates of specimen appearance

PROBLEM	CAUSE	SOLUTION
	During Cytocentrifugation	
Too few cells	Exit port misaligned with blotter, resulting in cell loss into blotter	Check alignment as seen through window from back of slide clip; seat blotter
	Cells lost through gap between exit port and blotter	Unlikely, though check assembled unit for alignment
	After Cytocentrifugation	
Too few cells	Suspension medium absorbed incompletely as a result of blotter becoming clogged by debris in previously non-centrifuged specimen and/or pores collapse from too much pressure from spring or excessive centrifugal force	Centrifuge specimen at 3000 rpm X 10 min. to sediment cells and leave debris in suspension to be discarded with supernatant Do not wet blotter before cytocentrifugation Cytocentrifuge specimen at 1000 rpm X 6-10 minutes
	Unabsorbed suspension medium can induce cell wash-off	Unload horizontal sample chamber, cell-side-up; let blotter absorb excess liquid; lift chamber and blotter away from slide; lay slide flat until thin film remains; immerse in fixative

b. Unusual Patterns of Cell Population Distribution

PROBLEM	CAUSE	SOLUTION
	Before Cytocentrifugation	
Crescent-shaped distribution	Cylinder filled incompletely Cells settle in cylinder during prolonged delay before cytocentrifugation	Fill cylinder completely Rapidly load sample chambers and immediately begin cytocentrifugation
Circular display area displaced away from label end	Slide not seated to foot of slide clip	Seat slide to foot of slide clip
Circular display area displaced toward label end	Slide placed in slide clip label-end down	Insert slide label-end up
Cells on underside of slide	Slides loaded backwards	Orient slide with labelled side facing exit port
	After Cytocentrifugation	
Cell population streams towards label end or to opposite end	Thinly layered cells too wet and are either pushed up the slide upon immersion in alcohol or slide down the slide following immersion	Let suspension medium evaporate nearly completely
Circular band of cells, acellular center, 'bull's-eye' distribution	Thickly layered cells, margins nearly dry but center remains very wet and promotes cell wash-off	Add less cell suspension

4.3 SUMMARY

To predictably produce cytocentrifuged preparations that exhibit within a 32 mm^2 circle a representative sample of randomly distributed, uncrowded, monolayered, flattened cells that are well preserved and well displayed, use the following recommended materials and methods and avoid those that are not.

a. Before Cytocentrifugation

DO:	DON'T:
Use unfixed fresh cell suspension	*Use cell suspensions collected in alcohol
Saponinize bloody cell suspensions	*Use bloody cell suspensions
Equalize differences in cell suspensions	*Cytocentrifuge unprocessed cell suspensions
Control the number of cells	*Estimate the number of cells
Use clean micro slides	*Use frosted or albuminized micro slides
Use balanced electrolyte solution	*Use normal saline
Add cell suspension to the tunnel	*Add cell suspension to the funnel
Keep cell suspension from blotter	*Let cell suspensions touch blotter
Fill sample chambers with comparable total volumes	*Fill sample chambers with markedly different total volumes

b. During Cytocentrifugation

DO:	DON'T:
Cytocentrifuge specimen at 1000 rpm for 3 to 4 minutes, until almost dry	*Cytocentrifuge specimen too rapidly, too lengthy, or too briefly

c. After Cytocentrifugation

DO:	DON'T:
Keep the cells slightly wet	*Let the cells air-dry, unless so intended
Immediately immerse the cytologic preparation in 95 percent ethanol for ethanol preps	*Let too much liquid remain over the cells
Allow the cellular monolayer to fully air-dry before staining when using Cytospin Collection Fluid	*Immerse in 95 percent ethanol until cell monolayers prepared with Cytospin Collection Fluid have sufficiently dried unless otherwise specified

5 CLEANING AND MAINTENANCE

5.1 CLEANING AND STERILIZING THE CYTOSPIN 3

All the components of the Cytospin 3 which are likely to become contaminated have been designed to be easily cleaned using mild detergent solutions, and then sterilized using a wide variety of agents.

In the interest of laboratory safety, Shandon recommends that suggested sterilization procedures are adhered to.

All the components of the sealed head, the centrifuge bowl liner, safety cover and front panel are designed to be easily cleaned.

Each component part is dealt with individually and, if the instructions are followed, no damage will occur. This is particularly important, since any chemical attack on the components of the sealed head or hinged safety cover will weaken the material and create a serious hazard.

When handling, cleaning or sterilizing any component, it is advisable to wear gloves as they offer protection against infection and chemical effects on the skin due to the detergent and/or disinfectants being used. After cleaning and sterilizing any component, it should be examined for damage. If in doubt of its condition, it should not be used on the centrifuge until inspected by your Safety Officer.

IMPORTANT NOTE

If you are in any doubt as to the type of disinfectant you are using—many brands do not declare the nature of the active ingredient—check with the manufacturer before using the solution with any component of the Cytospin 3.

You may use the disinfectant that you feel is effective in your laboratory. Be aware, however, that HYPOCHLORITES IN STRONG SOLUTION AND PHENOL WILL DAMAGE THE CYTOSPIN AND ACCESSORIES.

When properly used, a 10% solution of commercial bleach, e.g., Clorox® is not damaging to the sealed head and sample chamber assemblies.

Glutaraldehyde used as a 2% solution is an alternative disinfectant agent that will not harm the instrument's components.

But there are various commercial brands—see their instructions before using. Dilution is normally with 0.3 percent bicarbonate buffer at pH 7.0-8.0. Leave for approximately one hour to ensure sterilization, although 20 hours' exposure is needed to destroy spores of Bacillus subtilus I.

5.2 DAILY AND WEEKLY MAINTENANCE

a. Front Panel

FREQUENCY:	Weekly and after any spillage.
CLEAN:	Use warm soapy water to clean panel; DO NOT use abrasive powders, these will damage the surface of the panel.
	Use 2% glutaraldehyde prepared according to manufacturer's instructions, or a 10% solution of commercial bleach and water.
NOTE:	a. Never clean the front panel with xylene, toluene or similar solvents.
	b. The front panel can be polished if required with a liquid or spray furniture polish.

b. Hinged Safety Cover

FREQUENCY:	Daily and after any serious spillage.
CLEAN:	Use warm soapy water, DO NOT use abrasive powders, these will scratch the surface and make it more difficult to clean and also create a potential source of cross-contamination. Use 2% glutaraldehyde prepared according to manufacturer's instructions, or a 10% solution of commercial bleach and water.
NOTE:	DO NOT clean the hinged cover with any solvents—these could weaken the safety cover.

c. Bowl Liner

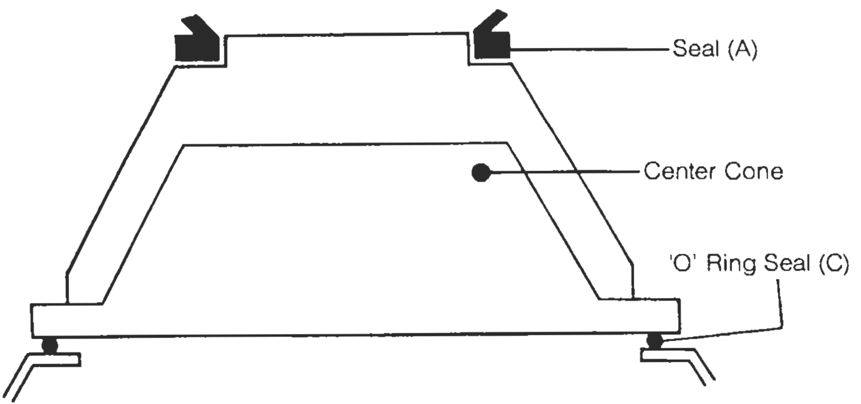
FREQUENCY:	Daily and after any serious spillage.
CLEAN:	Use warm soapy water, DO NOT use abrasive powders, they will create scratches which may harbour micro-organisms and make sterilization difficult. Use 2% glutaraldehyde prepared according to manufacturer's instructions, or a 10% solution of commercial bleach and water.

d. Sealed Head Assembly

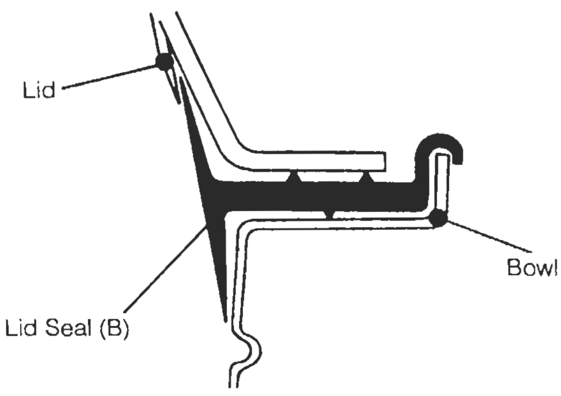
FREQUENCY:	Daily and immediately after any serious spillage.
STERILIZE:	The head assembly can be sterilized as a whole unit by autoclaving at 121°C (250°F) for 15 minutes. It is necessary to unlock the lid so that the pressure inside the head will be equalized with that in the autoclave and to allow the free entry of steam into the interior of the head. An alternative disinfecting method is to use 2% glutaraldehyde prepared according to manufacturer's instructions, or a 10% solution of commercial bleach and water.
CLEAN:	After sterilization of the whole head, all components should be removed, washed in warm soapy water and dried in an oven (maximum temperature 65°C or 149°F) before reassembly.

e. Sealed Head Base

FREQUENCY:	Daily and immediately after any serious spillage.
STERILIZE:	Autoclave at 121°C (250°F) for 15 minutes. Wipe with 2% glutaraldehyde, a 10% solution of commercial bleach and water, or a phenolic disinfectant prepared according to manufacturer's instructions.
CLEAN:	After sterilization clean using warm soapy water, rinse in clean water and dry. DO NOT use hard brushes to clean this component.



The Lid Seal (B) pulls off the raised rim of the bowl and the replacement clips on the rim (see below).



To replace the third seal a screwdriver is required. On the underside of the bowl, four (4) screws can be seen. Remove these and the center cone comes away from the bowl, exposing the 'O' Ring Seal (C). The replacement fits into the groove in the center cone, and the cone is then secured to the bowl using the four screws; ensure that the seal locates in the groove properly to avoid 'pinching' when tightening the screws.

j. Reusable Sample Chamber

FREQUENCY:	After use.
STERILIZE:	Autoclave at 121°C (250°F) for 15 minutes* or disinfect by immersing in 2% glutaraldehyde solution according to instructions, or a 10% solution of commercial bleach and water. DO NOT expose for periods longer than 5 minutes. DO NOT use phenolic disinfectants, these will attack and roughen the surface of the sample chamber which could reduce the cell recovery.
CLEAN:	Use a cotton-tipped applicator swab and hot soapy water to clean the outlet port and funnel. Rinse in clean water and dry. DO NOT use a wire brush. *Repeated autoclaving may cause discoloration of the sample chamber, but it will not affect the performance of the chamber. Do not autoclave the plastic cap.

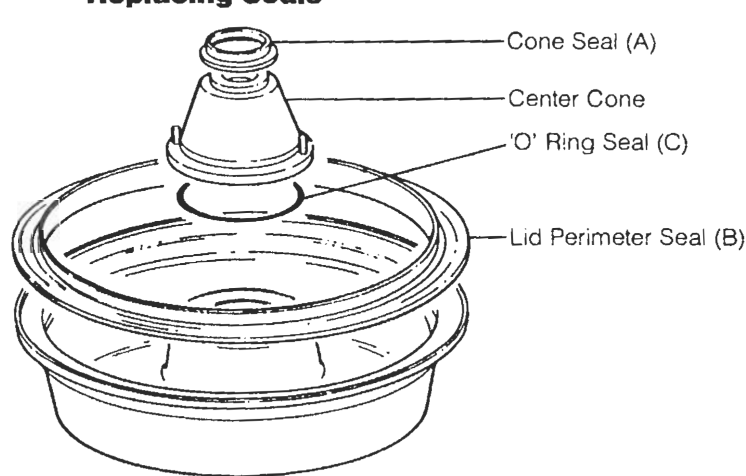
k. Filter Cards

These cards are disposable, single-use items and should be discarded into a phenolic disinfectant for sterilization before being disposed of.

l. Silicone Rubber Seals

These will normally be sterilized with the component to which they are fitted. The seals will eventually show signs of wear, stretching or chemical attack. It is advisable to replace all seals annually.

Replacing Seals



There are three (3) seals fitted to the Sealed Head. The Cone Seal (A) and the Lid Seal (B) need no tools for replacement; the Center Cone Seal (A) simply pulls off and the replacement stretches onto the collar on the center cone; ensure that the thin lip of the seal faces upwards.

f. Sealed Head Slide Clip Assembly Support Plate

FREQUENCY:	Daily
STERILIZE:	As Sealed Head—Base
CLEAN:	As Sealed Head—Base
NOTE:	This component is easily removed from the Sealed Head Base by removing two thumb screws. This enables the undersurface of the support plate to be cleaned easily and also facilitates cleaning of the inside surface of the sealed head base. The support plate can be totally immersed in 2% glutaraldehyde disinfectant if required.

g. Sealed Head Lid

FREQUENCY:	Daily
STERILIZE:	As Sealed Head—Base
CLEAN:	As Sealed Head—Base

NOTE:	The silicone rubber seal around the rim of the lid can be easily removed and replaced. It is advisable to remove this seal at weekly intervals to enable all the surface of the lid to be cleaned. A new seal should be fitted at intervals of 12 months. It is further recommended to apply any readily available lubricant (oil or grease) to the locking ball bearing at monthly intervals to ease removal of lid.

h. Cytoclip™ Slide Clip

FREQUENCY:	Daily
STERILIZE:	Autoclave at 121°C (250°F) for 15 minutes or disinfect by immersing in 2% glutaraldehyde solution according to manufacturer's instructions, or a 10% solution of commercial bleach and water.
CLEAN:	Rinse in clean water before use and hot air dry.
NOTES:	Prior to sterilization, it is advisable to release pressure from the spring of both the Cytoclip slide clip as well as the spring-loaded slide clip requiring the opener. For the Cytoclip slide clip (Cat. Number 5991052M), snap open the spring. For the spring-loaded slide clip (Cat. Number 5991017M), turn the pressure release screw fully counter-clockwise.

6 SPECIFICATIONS

6.1 TECHNICAL DATA

Power Requirements: 150VA

Fuse Sizes: 110V—2 Amp (Slow Blow)
240V—1 Amp (Slow Blow)

Sample Chamber Capacity: 0.1 ml—0.5 ml

Instrument Dimensions: (approximately)

Width	385 mm (15¼″)
Height	215 mm (8½″)
Depth	556 mm (21⅞″)
Clearance Height	560 mm (22″)
Weight	18 kg (39¾ lb) (Net)

Note: For instrument 'SUPPLY VOLTAGE RANGE' and 'SUPPLY FREQUENCIES' please refer to PARTS LIST Section 6.2

6.2 PARTS LIST

a. Cytospin 3, Cell Preparation System

DESCRIPTION	CATALOGUE NUMBER
200-250V, 50/60Hz	74000001
100-120V, 50/60Hz	74000003
100-120V, 50/60Hz (U.S.A)	74000002
200-250V, 50/60Hz (WEST GERMANY)	74000004
200-250V, 50/60Hz (FRANCE)	74000005

b. Accessories for Cytospin 3 (Sold Separately)

DESCRIPTION	CATALOGUE NUMBER
Cytoclip™ slide clip—stainless steel (Pack of 6)	59910052
Sample Chamber-autoclavable (1)	59910021
Sealed Head (slide clips not supplied)	59910018
Head Seals—silicone rubber (Pack of 3)	59910019
Cytospin 3 Operator Handbook (1)	74010120

c. Consumables for Cytospin 3 (Sold Separately)

DESCRIPTION	CATALOGUE NUMBER
Filter Cards (box of 200) thick, white for volumes of 0.5 ml	59910022
Closure Caps (Pack of 12)	59910025
Cytofunnel™ Disposable Sample Chambers with caps (box of 50)—white cards	59910040
Cytospin Cytoslide® Microscope Slides (box of 100)	59910051
Cytospin Collection Fluid (4 L container with pump)	67680001 (99900301-U.S.A.)
Cytospin Collection Fluid (case of 50 each, 120 ml cups half filled)	67680017 (99900321-U.S.A.)
Cytospin Collection Fluid (pack of 2 each 500 ml bottles)	— (99900315-U.S.A.)
Cytospin Collection Fluid (pack of 2 each 10 L bottles)	67680016 (99900310-U.S.A.)
Cell-Fixx™ spray fixative (50 ml bottle)	— (99900325-U.S.A.)
Cell-Fixx™ spray fixative (pack of 6 each 50 ml bottles)	99900326
Cytoblock™ Cell Block Preparation System	74010150

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HEALTH AND SAFETY AT WORK ACT (1974)

In common with all suppliers of instruments for use at work, we are obliged under the terms of the above act to advise our customers on the safe installation, operation and maintenance of our equipment.

Our instrumentation has been designed to accepted standards of safety and its use does not entail any hazard if used according to our instructions. However the following safety precautions should be observed:

(i) All personnel using the instrumentation should have read and understood the handbook and should operate the equipment in accordance with the instructions.

(ii) Voltages above 110V A.C. are present in the instruments and access covers should only be removed by trained Shandon service engineers, except where otherwise stated.

(iii) It is very important that normal laboratory standards of safety and good housekeeping are carried out and common sense rules applied.

(iv) All queries should be referred to our Service Department.

(v) Correct maintenance procedures are essential if the equipment is to be maintained in a safe working condition and it is recommended that a maintenance contract be taken out with our Service Department.

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