

## AEROSPRAY® CYTOLOGY

Slide Stainer/Cytocentrifuge MODEL 7522

USER'S MANUAL





# AEROSPRAY® Cytology Slide Stainer/Cytocentrifuge

**MODEL 7522** 

**USER'S MANUAL** 

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#### 1.1 Instrument Overview

#### **Using This Manual**

This manual provides instructions to install, operate, and maintain the Aerospray Cytology Slide Stainer/ Cytocentrifuge Model 7522. The manual is an important part of the product. Read it carefully and completely before setup and first use of the instrument.

If additional accident prevention and environmental protection requirements exist in the country of operation, this manual must be supplemented by appropriate instructions to ensure compliance.

#### **Safety Regulations**

This instrument has been designed and tested in accordance with safety regulations for electrical control, regulating, and laboratory instruments. In order to maintain this condition and ensure safe operation, the operator must observe all the instructions and warnings contained in this manual. For current information about applicable standards, please refer to the CE Declaration of Conformity included with the documents shipped with this instrument.

#### **Understanding Warnings**

This manual uses three warning levels to alert the user to important information as shown in the following examples.

#### **⚠** WARNING!

A Warning alerts to the possibility of personal injury, death, or other serious adverse reactions stemming from the use or misuse of this instrument or its components.

#### **⚠** CAUTION:

A Caution alerts to possible problems with the instrument associated with its use or misuse. Such problems include instrument malfunction, failure, damage, damage to the sample, or damage to other property. Where applicable, a Caution may include precautions to be taken to avoid the hazard.

**NOTE:** A Note reinforces or supplies additional information about a topic.

#### **Specific Warnings**

Pay particular attention to the following safety precautions. If these safety precautions are ignored, injury or damage to the instrument may occur. Each individual precaution is important.

#### WARNING!

Install the Cytology stainer in a well-ventilated area. If ventilation is inadequate, operate the instrument under a safety hood.

#### **⚠ WARNING!**

Reagents used in the stainer contain moderately hazardous chemicals that require care in handling. Always use appropriate safety measures including gloves and eye protection when handling reagents.

#### **♠ WARNING!**

Always wear protective clothing and eye protection when using SS-029 nozzle cleaning solution. Dispose of used solution properly.

#### **⚠** WARNING!

If power is lost while the stainer is running, the lid will remain locked until power is restored. Do not attempt to open the lid while power is off.

#### ♠ WARNING!

Electrical shock hazard: Do not open this instrument or attempt internal repairs. Refer servicing to qualified service personnel. Contact ELITechGroup Biomedical Systems service.

#### **↑** CAUTION:

This instrument has been designed and tested to CISPR 11 Class A and FCC Part 15 Class A. In a domestic environment it may cause radio interference, in which case, measures to mitigate the interference may be necessary. This instrument complies with the emission and immunity requirements described in the IEC 61326 series.

In an electromagnetic environment, the environment should be evaluated prior to operation of the device.

Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these may interfere with the proper operation.

#### **⚠** CAUTION:

To avoid serious instrument damage, always use reagents supplied by ELITechGroup. Using reagents not supplied by ELITechGroup may void the warranty.

#### **⚠** CAUTION:

Only spare parts supplied or specified by ELITechGroup should be used in this instrument. Using non-approved parts may affect the performance and safety features of the instrument. If the instrument is used in a manner not specified by ELITechGroup, the protection provided by the instrument may be impaired. If in doubt, contact your ELITechGroup representative.

#### Functional Description

The Aerospray Cytology Slide Stainer/Cytocentrifuge (Model 7522) is a dual-purpose, microprocessor-controlled slide staining and cell preparation system. In use, atomizing spray nozzles apply fresh reagents onto microscope slides prepared with cytological specimens. The slides are mounted in a rotating carousel for processing.

Staining options include a Progressive and Regressive stain mode with programmable stain settings for user customization.

#### Key Features

- · Minimized reagent consumption
- · Rapid staining
- · Barcode scanner for tracking specimens and reagents
- Reagent and specimen traceability
- · User traceability
- · Administrator password
- · Interactive touchscreen display

- Multiple languages
- High-volume staining productivity (12 or 30 slides per stain cycle)
- · Automatic Clean Cycle to purge the reagent spray nozzle with approved alcohol
- · Separate reservoir, delivery tube, and pump for each reagent
- · Stain sequence programmability
- · Reagent and waste level monitoring
- · Log files

The correct accessory must be used for each function. The Cytopro® Cytocentrifuge Rotor is available as an option offering additional features (see Section 8).

#### Intended Use

The Aerospray® Cytology Slide Stainer/Cytocentrifuge Model 7522 is intended for use by laboratory professionals to stain cytology specimens using the Papanicolaou staining method with ELITechGroup Aerospray® Cytology Papanicolaou stains only. The optional Cytopro® rotor allows preparation of slides by cytocentrifugation before staining.

Table 1: General Specifications

Category	Characteristics		
Slide Carousel Capacity	1 to 12 or 1 to 30, depending on carousel		
Carousel Rotation Speed	From 10 to 1000 rpm (pre-programmed)		
	Accuracy: 10 rpm to 99 rpm ± 2 rpm 100 rpm to 1000 rpm ± 5%		
Cytocentrifuge Rotor Speed	100 to 2000 rpm (± 5%), user programmable		
Stain Consumption	Refer to Approximate Stain Consumption, Table 4		
Operating Time	Refer to Run Time Sequence, Table 13		
Display	7 in. LCD WVGA (800 x 480 pixels) TFT		
Touch Screen Controls	Numeric and alpha-numeric programming keys		
Drain Connection	Connector on rear panel accepts male connector attached to vinyl drain tube.  1.8 meters (6 ft.) length supplied		
Ventilation	Air is exhausted from the stainer via a female ½ inch SAE pipe thread fitting		
Dimensions			
Width	57 cm (22 in.)		
Depth	54 cm (21 in.)		
Height (lid closed)	25 cm (10 in.)		
Height (lid open)	58 cm (23 in.)		
Weight	18 kg (~39.7 lb.) unpacked 19.6 kg (~43.2 lb.) packed		
Electrical Requirements	100 to 240 VAC (± 10%) @ 50 to 60 Hz		
Power Consumption	200 VA		

Category	Characteristics
Over Current	Fuses (Qty 2): T2A250V
Ambient Temperature	
Operating	15 to 30 °C (59 to 86 °F)
Storage	-10 to 50 °C (14 to 122 °F)
Relative Humidity	≤ 80% non-condensing
Altitude	≤ 2000 m (≤ 6562 ft.)
Pollution Degree	2
Heat Dissipation	
Maximum	512 Btu/hour
Average During Staining	102 Btu/hour
Average While Idle	41 Btu/hour
Maximum Sound Emission	Adjustable; maximum 60 dB (SPL) intensity  @ 1m and < 80 dB. (Typical -72 dB)

Table 2: Performance Specifications

Category	Characteristics		
Reagent Spray Nozzles	Two reagent nozzles: ABCE (which controls these reagents under microprocessor control) and DF (hematoxylin)		
Reagents	A – SS-051A (EA-50 Stain)		
	B – SS-051B (Orange G Stain)		
NOTE: Use only ELITechGroup reagents. REF numbers	C – SS-051C (Bluing Agent)		
for this stainer include the following: SS-051A, SS-051B,	D – SS-051D or SS-051D2 (Hematoxylin Stain)		
SS-051C, SS-051D, SS-051D2, SS-051E, and SS-051F.	E – SS-051E (Alcohol Wash)		
See Appendix A for detailed information about reagents.	F – SS-051F (Acid Alcohol Wash)		
Stain Program Settings	Two example programs: Progressive 1, Regressive 1		
	Number of specimen slides to stain: Selectable from main screen – 4, 6, 8, 12, 16 or Full		
	Stain modes: Progressive or Regressive.		
	Intensity settings: 0-9 within each stain mode		
	Staining Customization: Each setting adjustable from 0-9		
	Nuclear/Htox (Hematoxylin)		
	Orange G		
	Bluing		
	Cytoplasmic/EA		
	Prewash Alcohol, Prewash Bluing		
	Acid Alcohol Wash, Bluing Wash, Orange G Wash, Final Wash		
	End Spin		

#### Table 3: Carousel and Rotor Information

Only the following slide staining carousels or cytocentrifuge rotor can be used in this instrument. Each should be used following the instructions in this manual or the Cytopro Applications Manual (RP-517).

Rotor/Carousel	Maximum RPM	Maximum Capacity	Maximum Sample Volume
12-Slide Carousel (AC-195)	1000 rpm	12 each, 26 mm x 76 mm (1 x 3 inch) microscope slides	N/A
30-Slide Carousel (AC-196)	1000 rpm	30 each, 26 mm x 76 mm (1 x 3 inch) microscope slides	N/A
Cytopro Cytocentrifuge Rotor (AC-160)	2000 rpm	8 each, standard chambers, plus slides	Up to 600 μL*
		8 each, Cytopro Magnum chambers, plus slides	Up to 6 mL*

<sup>\*</sup>Do not overfill cytocentrifuge chambers. See Cytopro Applications Manual or Methods Manual for detailed instructions and warnings.

#### Table 4: Approximate Stain Consumption (mL)

The following table provides an estimate of the volume of stain used per staining cycle when running a full 12-slide carousel when running the default settings programed on the instrument.

**NOTE:** The values in parentheses provide an estimate of the volume of stain used per staining cycle when running a full 30-slide carousel.

Stain	Stain Mode		Cleaning Mode		
Stairi	Progressive 1	Regressive 1	System Clean	Carousel Clean	
Stain A (EA-50)	12.8 (23.4)	20.5 (38.7)	0.0	0.0	
Stain B (Orange G)	11.2 (20.3)	19.0 (35.6)	0.0	0.0	
Stain C (Bluing Agent)	10.6 (21.1)	16.8 (33.4)	0.0	0.0	
Stain D (Hematoxylin)	12.8 (23.7)	24.0 (45.8)	0.0	0.0	
Stain E (Alcohol)	30.3 (60.8)	51.4 (102.6)	3.0	0.0	
Stain F (Acid Alcohol)	4.1 (5.6)	8.7 (14.7)	3.4	7.5	

Table 5: Explanation of Symbols

Symbol	Standard Reference	Standard Title	Symbol Title	Symbol Meaning
$\sim$	IEC 60601- 1 Reference no. Table D1, Symbol 8 (IEC 60417-5032)	Medical electrical equipment — Part 1: General requirements. for basic safety and essential performance	Alternating current	To indicate on the rating plate that the equipment is suitable for alternating current only; to identify relevant terminals
EC REP	ISO 15223-1: 2021 Reference no. 5.1.2	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Authorized Representative in the European Community/ European Union	Indicates the authorized representative in the European Community / European Union
CH REP	MU600_00_016e V3.0	Information Sheet Obligations Economic Operators CH	Swiss Authorized Representative	Indicates the authorized representative in Switzerland

Symbol	Standard Reference	Standard Title	Symbol Title	Symbol Meaning
LOT	ISO 15223-1: 2021 Reference no. 5.1.5. (ISO 7000-2492)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified. Synonyms for "batch code" are "lot number", "lot code" and "batch number".
	ISO 15223-1:2021 reference no. 5.4.1 (ISO 7010 – W009)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Warning; Biological hazard	Bio-contamination warning: Use care when operating upper cooling system and initiation needle.
REF	ISO 15223-1: 2021	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Catalogue number Catalog number	Indicates the manufacturer's catalog number so that the medical device can be identified ISO 15223 Catalogue number ISO 7000 Catalog number
$\triangle$	ISO 15223-1: 2021 Reference no. 5.4.4. (ISO 7000-0434A)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Caution	To indicate that caution is necessary when operating the device or control close to where the symbol is placed, or to indicate that the current situation needs operator awareness or operator action in order to avoid undesirable consequences
C€	EU 2017-746 Reference no. ANNEX V	REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/ EEC and 2010/227/EU	CE marking	(43) 'CE marking of conformity' or 'CE marking' means a marking by which a manufacturer indicates that a device is in conformity with the applicable requirements set out in this Regulation and other applicable Union harmonization legislation providing for its affixing
Ţ <u>i</u>	ISO 15223-1:2021 Reference no. 5.4.3. (ISO 7000-1641)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use
2	ISO 15223-1:2021 Reference no. 5.4.2. (ISO 7000- 1051)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Do not re-use	Indicates a medical device that is intended for one single use only NOTE: Synonyms for "Do not reuse" are "single use" and "use only once".
<b>®</b>	ISO 15223-1: 2021 Reference no. 5.2.8. (ISO 7000-2606)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Do not use if package is damaged and consult instructions for use	Indicates a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for additional information
, <b>T</b>	ISO 15223-1: 2021 Reference no. 5.3.1. (ISO 7000-0621)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Fragile, handle with care	Indicates a medical device that can be broken or damaged if not handled carefully
	IEC 60417-1 Reference no. ISO 7000-5016	Graphical symbols for use on equipment	Fuse	To identify fuse boxes or their location
4	IEC-TR-60878 Reference no. ISO 7000-1135	Graphic symbols for use on electrical equipment in a medical practice	General symbol for recover/recyclable	To indicate that the marked item or its material is part of a recovery or recycling process
IVD	ISO 15223-1:2021 Reference no. 5.5.1.	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	In Vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device

Symbol	Standard Reference	Standard Title	Symbol Title	Symbol Meaning
类	ISO 15223-1: 2021 Reference no. 5.3.2. (ISO 7000-0624)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Keep away from sunlight	Indicates a medical device that needs protection from light sources
<b></b>	ISO 15223-1: 2021 Reference no. 5.1.1. (ISO 7000-3082)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Manufacturer	Indicates the medical device manufacturer
X	DIRECTIVE 2012/19/EU (WEEE)	N/A	Collect separately	Separate collection for waste of electrical and electronic equipment. Do not dispose of battery in municipal waste. The symbol indicates separate collection for battery is required
	DIRECTIVE 2002/96/EC (WEEE)	N/A	Waste stream disposal status	Do not dispose of electronic equipment in general waste stream
6	N/A	N/A	Open bottle stability	Indicates a reagent is stable after opening for the number of months specified
SN	ISO 15223-1: 2021 Reference no. 5.1.7. (ISO 7000-2498)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Serial number	Indicates the manufacturer's serial number so that a specific medical device can be identified
1	ISO 15223-1: 2021 Reference no. 5.3.7. (ISO 7000-0632)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed
$\square$	ISO 15223-1: 2021 Reference no. 5.1.4. (ISO 7000-2607)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Use by date	Indicates the date after which the medical device is not to be used
<u> </u>	iso_grs_7010_WOO1	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	General warning sign	To signify a general warning
	GHS02	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Flammable	Medical device contains materials that are flammable. Appropriate caution should be taken
<b>(b)</b>	GHS03	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Oxidizing	Medical device contains materials that are oxidizing. Appropriate caution should be taken
	GHS05	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Corrosive	Medical device contains materials that are corrosive. Appropriate caution should be taken
	GHS06	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Toxic	Medical device contains materials that are toxic. Appropriate caution should be taken

Symbol	Standard Reference	Standard Title	Symbol Title	Symbol Meaning
<u>(!)</u>	GHS07	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Harmful	Medical device contains materials that are harmful. Appropriate caution should be taken
	GHS08	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Health Hazard	Medical device contains materials that are a health hazard. Appropriate caution should be taken
¥z	GHS09	Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Eighth Revised Edition	Environmental Hazard	Medical device contains materials that are an environmental hazard. Appropriate caution should be taken
<b>50</b>	N/A	Administrative Measure on the Control of Pollution Caused by Electronic Information Products (China)	Environment Friendly Use Period	Indicates the period of time before any RoHS substances are likely to leak out causing harm to the environment.
	N/A	N/A	Do not use pumps	Indicates products are to be used for manual cleaning only. Do not pump the product through instrument.
	ISO 15223-1: 2021 Reference no. 5.3.8. (ISO 7000-2620)	Medical devices — Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.	Humidity limitation	Indicates the range of humidity to which the medical device can be safely exposed
UK	N/A	https://www.gov.uk/ guidance/using-the- ukca-marking#when-to- use-the-ukca-marking	UKCA Mark	UK product marking that is required for medical devices being placed on the marketing in Great Britain.

## 1.2 Instrument Description

Figure 1: Front and Right-Side Panels



- 1. 30-Slide Carousel
- 2. 12-Slide Carousel
- 3. Lid with Safety Lock
- 4. Bowl
- 5. Front Panel with Touch Screen Display
- 6. Right Side Panel with Label Indicating Reagent Positions:
  - A. EA-50 Stain
  - B. Orange G Stain
  - C. Bluing Agent
  - D. Hematoxylin Stain
  - E. Alcohol Wash
  - F. Acid Alcohol Wash (bottle is off tray)
- 7. Reagent Tray

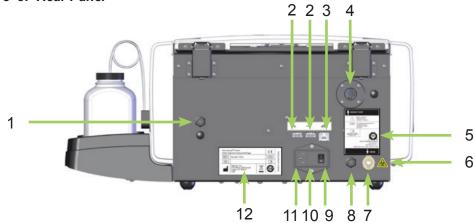
Figure 2: Front Panel and Touchscreen



- 1. Standby/Ready Button
- 2. Touch Screen

The front panel features an interactive touchscreen display. Refer to Touchscreen and User Interface (Section 1.3, Table 7) for more information.

Figure 3: Rear Panel



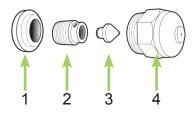
- 1. Level Detect Connection for Reagent F (Acid Alcohol Wash)
- 2. USB Ports
- 3. Network Ethernet Connection
- 4. Exhaust Vent
- 5. Rear Panel Label
- 6. Biohazard Warning Label
- 7. Waste Tube Connection
- 8. Level Detection Connection for Waste Container
- 9. Power Switch
- 10. Fuse Door
- 11. Power Cord Connection
- 12. Model/Serial Number Label

Figure 4: Stainer Bowl Components



- 1. Nozzle DF (Reagents D Hematoxylin Stain, F Acid Alcohol Wash)
- 2. Nozzle ABCE (Reagents A EA-50 Stain, B Orange G Stain, C Bluing Agent, E Alcohol Wash)
- 3. Drive Hub

Figure 5: Nozzle Diagram



- 1. Mixing Insert
- 2. Compression Screw
- 3. Swirl Cone
- 4. Nozzle Housing

Table 6: Preventive Maintenance Kit (Catalog Number AC-184)

Component	Name	Description
	Manual Priming Tool	Primes air-locked pumps.
CM <sup>2</sup>	Silicon Grease	Lubricates the nozzle threads for easy assembly.
Appendix	Nozzle Wire	Cleans nozzle housing orifices.

Component	Name	Description
	Nozzle Cleaning Strainer	Strains the nozzle parts to prevent them from going down a drain.
	Nozzle Tool	Unscrews nozzles from the stainer bowl.
	Nozzle Wrench	Disassembles the nozzle.
	Nozzle Brush	Cleans nozzles without removing them from the stainer.
	Volume Test Collection Tubes (small tube)	Collects reagents while performing the Volume Test.
	Nozzle Maintenance Tube Stand	Holds Nozzle Cleaning Tubes (large tube) and Volume Test Tubes (small tube).
	Nozzle Cleaning Tubes (large tube)	For soaking nozzles in the Nozzle Cleaning Solution.

#### Barcode Reader

An optional barcode reader is available for the Cytology Stainer/Cytocentrifuge (Model 7522).

Figure 6: Barcode Reader



#### Other Needed Items

The following parts are not available worldwide from ELITechGroup, but they can be obtained locally:

• Approved alcohol (Ethanol or Isopropanol)

Empty bottles are available from ELITechGroup.

#### **A** CAUTION:

ELITechGroup does not provide approved alcohols. They should be purchased locally, observing the recommendations for safety and chemical risk on the Safety Data Sheet (SDS).

## 1.3 Touchscreen and User Interface

Users control all instrument functions from the interactive touchscreen display.

Table 7: Front Panel/Main Screen Function Keys

Button	Name	Description
	Standby/Ready	With instrument power ON:
		Blue = Ready
		Amber = Standby
		Pressing standby runs a System Clean Cycle and places instrument into standby mode.
		The Standby/Ready button also accesses the touchscreen calibration function. Refer to System Setup Menu (Section 3.1).
	Maintenance	Accesses features for verifying proper nozzle performance and places pumps in a testing sequence. Accesses the line priming, Pattern Test, Volume Test, and Line Flush functions.
	System Clean/ Carousel Clean	Performs the selected Clean Cycle. System Clean (left button). Carousel Clean (right button).
	Cyto	Enters the Cytocentrifuge mode.
1	System Information	Shows the system information, including serial number and software version. Allows access to the System Setup features. Refer to System Setup Menu (Section 3.1).
?	Help	Opens the software Help file.
	Programs	Allows users to select or edit programs.
-	Start/Load Slides	Starts a Stain or Cytocentrifuge cycle. Button is inactive until a program is created. Refer to Creating a Stain Program (Section 3.1).
		With Slide Tracking enabled, opens the Scan and Load Slides menu (Section 3.2).
	Number of Specimen Slides	Selects the number of specimen slides in the carousel. Users staining an odd number of specimen slides should press the next higher specimen slide number icon.
	Back	Returns to the previous menu.
×	Stop	Aborts any operation.
<b>/</b>	ОК	Indicates completion of current task.

Button	Name	Description
255	System Setup	Allows users to modify the software settings.
		See System Setup Menu (Section 3.1).

Table 8: System Setup Keys

Button	Name	Description
	Stain Programs	Allows users to create, edit, and delete stain programs.
	Cyto Programs	Allows users to create, edit, and delete cytocentrifuge programs.
	Reagents	Allows users to edit reagent information.
	Users	Allows users to create and change user accounts.
$\checkmark$	QC/Maintenance Tracking	Enables slide tracking, preventive maintenance tracking, and reagent tracking.
	Level Detect	Allows users to manage the automatic reagent level detection system.
	Language	Allows users to change the display language.
	System Log	Allows users to control logging functions.
	Network Settings	Allows users to change network settings.
	Beeper	Allows users to change audible alerts.
31	Set Date/Time	Allows users to set the date and time.
5	Restore Defaults	Restores programming to default settings.
<b>-</b>	Login	Enters Login sequence for authorized users.
	Logout	Logs authorized users out. Users must log in again to use the stainer.
	Save	Saves the entered or selected information.
+	Add	Enters programming mode for creating staining and cytocentrifuge programs. Also allows the system administrator to authorize new users. Allows manual entry of slide or specimen information.

Button	Name	Description
	Delete/Erase/Remove	Deletes or erases the selected item.
	Edit/Change User	Allows editing of an existing stain or cytocentrifuge program. Allows manual entry of slide or specimen information (stain or cytocentrifuge mode). Also allows system administrator to edit user information.
$\phi$	Zero	Zeros the Level Detect sensors.
	Calibrate	Calibrates the Level Detect system.
	Unselected	Shows an unselected option.
	Selected	Shows a selected or enabled option.

Table 9: Maintenance Function Keys

Button	Name	Description
	Individual Prime Buttons (A, B, C, D, E, F)	Primes the selected line.
0)	ABCDEF Prime button	Primes all (A, B, C, D, E, F) reagent lines simultaneously.
	Pattern Test	Performs Pattern Test to ensure nozzles are clear of debris and spraying properly.
	Volume Test	Performs Volume Test to verify the selected nozzle volume is within the correct range.
	Line Flush	Performs Line Flush function for A, B, and D reagent lines.
•	60-Sec Prime	Runs the pumps for 1 minute to prime the lines.
	QC/PM	Shows the Preventive Maintenance and Quality Control logs, when enabled from the System Setup menu (Section 3.1).

## 2.1 Instrument Setup

#### Unpacking and Installing the Stainer

Follow this sequence when using this instrument for the first time. Details about these operations are given in the next three sections.

- · Install the drain tube
- Plug in the power cord and switch the power ON (I)
- · Install all reagent bottles
- Install the barcode reader (optional)
- · Prime all reagent lines
- · Perform the Clean Cycle
- · Zero the automated reagent level detect sensors
- · Perform the Hub Pattern and Spray Volume tests

#### **A** CAUTION:

Contact ELITechGroup before installing the instrument if there appears to be any damage to the packaging or instrument.

- 1. Unpack and inspect the instrument.
- 2. Check that the contents of the boxes match the packing lists for the instrument and accessories.
- 3. Open the instrument lid and remove the cardboard tube that protects the hub.

**NOTE:** Keep the box and packaging material to repack the instrument if service is required by the manufacture.

4. Place the instrument on a flat surface, free from dust and vibration and away from direct sunlight.

**NOTE:** Position the instrument with the rear panel at least 30 cm (12 in.) from obstructions or hazardous materials.

#### Connecting the Drain Tube and Waste Container



- 1. Insert the waste tube connector into the rear panel receptacle until a click is heard.
- 2. Ensure the waste tube has no loops or kinks and is as straight and as short as possible. Cut off excess tubing as needed.

#### **A** CAUTION:

Keep the drain tube straight and as short as possible. The maximum length is 1.8 m (72 in.). The waste container must be positioned lower than the stainer.



- 3. Connect the drain tube to the waste container.
- 4. If using a waste bottle with level detect (AC-182):
  Connect the waste monitoring cable to the rear panel receptacle.
- 5. Connect the waste monitoring cable to the waste container lid.

#### **Connecting Power**

**NOTE:** Use a surge protector to isolate the instrument from electrical spikes and surges.

- 1. Make sure the power switch is **OFF (O)**.
- 2. Plug the power cord into the power connector on the rear panel of the instrument.
- 3. Plug the power cord into a properly rated AC electrical outlet.
- 4. Turn the power switch **ON (I)**. After a brief delay the Main menu will appear.



Installing Standard 500 mL Reagent Bottles for the First Time

#### **⚠** WARNING!

Reagents used in the instrument contain moderately hazardous chemicals that require care in handling. Always handle reagents using appropriate safety measures, including gloves and eye protection.



**NOTE:** Reagents should be stored according to the conditions specified on their label. After opening, reagents are stable for 90 days unless otherwise indicated by the symbol shown at left.

1. Place each 500 mL reagent bottle in the correct position.



- A. SS-051A EA-50 Stain
- B. SS-051B Orange G Stain
- C. SS-051C Bluing Agent
- D. SS-051D or SS-051D2 Hematoxylin Stain
- E. SS-051E Alcohol Wash
- F. SS-051F Acid Alcohol Wash (off tray)

NOTE: See Appendix A for complete identification of all reagents used in this stainer.

#### **A** CAUTION:

To avoid severe damage, never use reagents containing organic solvents in this instrument, unless supplied by ELITechGroup, or specified in official ELITechGroup formulation instructions.

**NOTE:** Immediately remove spills in the reagent tray to preserve the accuracy of the reagent level detecting system.

- 7. For all reagents using the standard 500 mL bottles:
  - ♦ Open a new bottle of reagent.
  - Record the reagent letter on each cap and retain for future use (such as long-term storage).
  - ♦ Remove caps from bottom of the dip tube, insert the corresponding dip tube and bottle adapter into the reagent bottle, install and tighten the ring cap.
- 8. For reagents F:
  - ♦ Open a new 500 mL bottle of reagent SS-051F.
  - ♦ Pour the contents from the 500 mL SS-051F bottle into the external reagent bottle with level detect (AC-197).
  - ♦ Insert the level detect sensor into the reagent bottle and tighten the bottle cap.
  - From the stainer side panel, connect the reagent F tubing to the barb fitting on the top of the external bottle.





Onnect the level detect cable to the external bottle and to the connector on the back of the instrument towards the side with the reagent tray.

#### Installing the Barcode Reader

A barcode reader can be connected to the stainer for scanning reagent bottles and specimen slides that contain barcodes. This allows easy reagent and specimen information tracking. If a barcode reader is not installed, reagent and specimen information can be entered manually (Section 3.2).

- 1. Place the barcode reader and stand on a level surface near the stainer.
- 2. Plug the barcode reader into the *left* USB port on the rear panel of the stainer. See Section 3.2 for instructions on using the barcode reader.



#### **Preparing the Stainer for Operation**

#### **Priming Procedures**

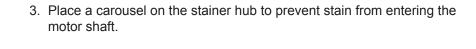
**NOTE:** The instrument is shipped with alcohol in the reagent lines. For proper performance, this alcohol must be replaced with the correct reagent for each reagent line prior to use.

Thoroughly purge and prime each reagent delivery line using the following instructions.



- 1. Remove each spray nozzle with the provided nozzle tool by turning counterclockwise.
- 2. Note the location of each nozzle so it can be returned to the original position during reassembly.







#### **⚠** CAUTION:

Fluid from priming can flood and damage the motor if the drain tube is not properly installed.



4. Press **Maintenance** from the Main menu.



5. Press the **A** prime button. Stain should appear within 10 seconds. When properly primed, a steady stream of reagent (no sputtering or breaks) flows from the nozzle receptacle.



- ♦ If stain appears, proceed to the next step.
- ♦ If stain does not appear within 10 seconds, perform the manual priming procedure in Section 6.3.

#### **↑** CAUTION:

Never operate a dry pump for more than 10 seconds. Operating a dry pump may cause damage to the instrument.

- 6. Repeat the previous steps for each reagent, (B, C, D, E and F).
- Press 60-Sec Prime to prime each reagent line with 200 mL of reagent to remove all the alcohol from the reagent lines and pumps. (Requires pressing the 60-Sec Prime button at least 2-3 times to prime each line sufficiently.)
- 8. Choose one of the following:
  - For initial setup, press ABCDEF to prime all lines simultaneously. (Requires pressing the ABCDEF prime button at least 2-3 times to prime each line sufficiently.)



♦ To prime individual lines, press the appropriate individual prime button (A, B, C, D, E, F). The pumps will run for 60 seconds and prime the selected lines.



- 9. Return the nozzles to their original positions and tighten clockwise with the nozzle tool.
- 10. With the nozzles installed, repeat Steps 5 and 6. A fine cone of spray should come from each nozzle.
- 11. After verifying nozzle performance, run the Clean Cycle (see below).



#### The Clean Cycle

Two Clean Cycles are available from the Main menu:



**System Clean** – cleans nozzle ABCE by purging it with SS-051E (Alcohol Wash) and cleans nozzle DF by purging it with SS-051F (Acid Alcohol Wash) while cycle progress is displayed on the screen. The cycle may be stopped at any time during the process.

**NOTE:** Pressing Standby/Ready performs a System Clean Cycle before the instrument goes on standby.



**Carousel Clean** – cleans the carousel and nozzle DF by purging with SS-051F (Acid Alcohol Wash). This clean cycle is of sufficient length to clean both nozzle DF and the carousel. The cycle may be stopped at any time during the process.

To run either Clean Cycle:

1. Place an empty carousel in the instrument and close the lid.

#### **A** CAUTION:

Never place any carousel loaded with specimens in the instrument for a Clean Cycle (including placing the instrument in standby mode). Specimens will be damaged if they contact reagents sprayed from the nozzles when pressing Clean or Standby.



2. Press Clean.

**NOTE:** Pressing Stop during the Clean Cycle causes the Incomplete Clean message to be displayed. Press **Clean** to complete the interrupted cycle.



- 3. Open the lid and remove the carousel when the Clean Cycle is complete.
- 4. Spray the interior of the bowl with 70 to 100% methanol, ethanol or SS-029 nozzle cleaning solution. Wipe the stainer bowl dry with paper towels.

**NOTE:** Perform the Storing the Instrument procedure (Section 5.2), if the instrument will remain idle for more than one week.

**NOTE:** At the end of a Clean Cycle, nozzle DF is filled with SS-051F (Acid Alcohol Wash) and nozzle ABCE is filled with SS-051E (Alcohol Wash), which remains in the nozzles. These are primed out of the nozzles during the first steps of a stain cycle.

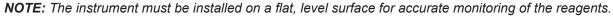
#### **Performing Tests**

The Pattern Test and Volume Test must be performed before using the instrument. See Section 6, Nozzle Maintenance and Performance.

#### Reagent Level Monitoring



Reagent Level Detect monitors reagent levels and sounds an alert when the reagent is running low, or when the waste container is full (when using the waste container with level detect). Reagent and waste container monitoring can be turned ON or OFF from the Level Detect menu. The system default is on for reagent monitoring and off for waste container monitoring.



Disable the Level Detect function for any line not using the standard 500 mL bottle.



#### **⚠** CAUTION:



This system is designed to warn when the reagent level is getting low. The instrument will continue running through these warnings. The user must monitor and replenish the reagent before running a stain cycle.

#### Enabling/Disabling Reagent Level Detect

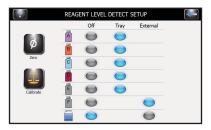


- 1. Press Information from the Main menu.
- 2. Press System Setup.



3. Press Level Detect. The display shows:





4. Press **Tray** to enable, or **Off** to disable a reagent line. Functions are grey when unselected, blue when selected. Press External to enable the external level detect for the F reagent line. Press External to enable level detect for the waste bottle.

NOTE: Must have external level sensors installed for instrument to detect the external bottles.

5. When finished, press Back to exit to System Setup menu.

#### Zeroing the Reagent Level Sensors

The Level Detect function must be zeroed at initial setup, when the stainer is moved, or if the level detect is not reporting correctly. If zeroing does not correct the problem, recalibrate the Level Detect function (Section 7.3).



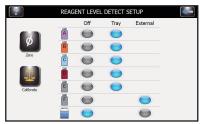
1. Press Information.



Press System Setup.



3. Press **Level Detect** to enter the Reagent Level Detect Setup menu:



**NOTE:** The stainer should be turned ON for at least 30 minutes before zeroing to stabilize level sensors. The instrument can be used during this time.



4. Press Zero. The display shows:



5. Remove all reagent bottles and press **Start**. The display shows:



NOTE: Vibrations or bumps to the instrument or lab bench can cause inaccuracies in zeroing or calibration.

- 6. After zeroing, press **OK**. Press the **Back** button to exit to the System Setup menu.
- 7. Return the reagent bottles to their correct positions in the tray.

**NOTE:** For accurate reagent level detection and calibration, dip tubes must follow their pre-formed coiled shapes.



## 3.1 System Setup Menu

Many software settings can be controlled from the System Setup menu, including:

- · Creating, editing, and deleting stain programs
- · Creating, editing, and deleting cytocentrifuge programs
- · Tracking reagent information
- · Managing user accounts
- · Enabling tracking features for slides, preventive maintenance, and reagents
- · Managing reagent level sensing
- · Changing the display language
- · Viewing and exporting the system log
- · Changing beeper settings
- · Setting the date and time
- · Restoring default settings

#### Accessing the System Setup Menu



1. Press System Information from the Main menu.



2. Press System Setup.



#### Stain Programs



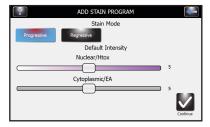
Stain Programs allows the user to create, edit, or erase custom staining programs according to the user's specific staining requirements. Programming parameters are governed by the stain mode used.

The stainer comes programmed with one default-staining program. Experiment with factory-programmed settings and adjusting or creating new programs according to need. See Appendix D, Stain Modes and Programming Options for information.

#### Creating a Stain Program

1. From System Setup, press Stain Programs. Depending upon how many stain programs have been added, an existing program may need to be erased to remain within the 12-program limit.



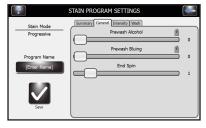


- 2. Press Add.
- 3. Select the desired Stain Mode (Progressive or Regressive) and Nuclear/Htox and Cytoplasmic/EA Intensity, then press Continue.

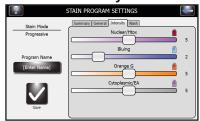
**NOTE:** Staining programming options are determined by the chosen stain mode. See Appendix D for additional information.



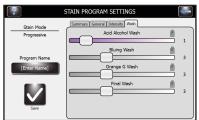
4. Select the **General** tab and set the desired program settings.



5. Select the **Intensity** tab and set the desired program settings.



6. Select the **Wash** tab and set the desired program settings.



- 7. Select **Enter Name** button and enter the desired name using the keypad.
- 8. Press Enter on the keypad.
- 9. Press Save.

#### Editing, Renaming, or Adjusting Stain Programs

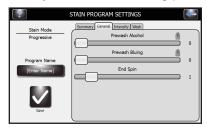
1. From Stain Programs menu select the program to be modified.

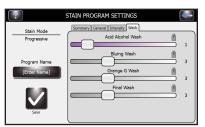


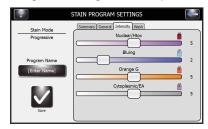
2. Press Edit.



- 3. Select the General, Intensity, or Wash tab.
- 4. Modify the desired staining parameters using the sliding tabs and press **Save**.







5. To rename the program, select **Program Name** and enter a new program name using the keypad.



- 6. Press **Enter** on the keypad.
- 7. Press Save.

See Appendix D for more information.

#### Administrator and User Accounts

One Administrator account and multiple (up to 50) user accounts can be created. The Administrator controls access to the system by adding and editing user accounts. Users cannot edit System Settings unless permitted by the administrator.

#### Creating an Administrator Account

1. From System Setup, select Users.



2. Select Lock System Setup Access.



3. Enter a password for the Administrator account (at least 4 characters) and press Enter.



4. Re-enter the password and press **Enter** to confirm.



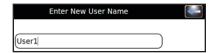
#### **Creating User Accounts**

NOTE: This function is available only if an administrator account has been created.

- 1. Press System Setup.
- 2. Enter the Administrator password and press **Enter**.
- 3. Press Users to reveal the Manage Users menu.
- 4. Select Enable Global Login.



- 5. Select Add User.
- 6. Enter a new user name.



- 7. Press Enter.
- 8. Enter a numeric passcode (at least 4 numbers) for the user account.



- 9. Press Enter.
- 10. Re-enter the passcode to confirm.



11. Press Enter.

#### Managing User Access

From the Manage Users screen, the Administrator has several options to manage user access to the instrument.



• Enable Global Login allows users to log in to the instrument. Users will log out manually or automatically (with user-selectable time options). See User Login/Logout below.

- Enable Run Login requires the current user to enter a password to run a Stain or Cytocentrifuge cycle. Global Login must be enabled to use this option.
- User System Access enables complete control of the instrument, including changing the System Setup options. This option can be controlled on an individual user basis, if Global Login is enabled.

#### **User Login/Logout**

With System Access locked and Global Login enabled, users must log in to use the stainer:

1. Select the User ID (User 1 in this example) and select a time from the Logout After Idle drop-down menu.

NOTE: Users can select how long the stainer can be idle before automatically logging the user out.

2. Press Login.



- 3. Enter the correct passcode for the selected user and press Enter.
- 4. Control returns to the Main screen and the stainer is ready for programming and staining.
- 5. Once Login is complete, the stainer advances to the Main screen. A Logout button and the username appear at the top right of the Main screen. Users can logout manually by pressing the logout button.



#### **Using Reagent Information Tracking**

Reagent information can be entered to help track reagent usage and expiration. Reagent information includes reference number, expiration date, lot number, date and time the reagent was last installed.



- 1. From System Setup, select QC/Maintenance Tracking.
- 2. Select **Enable Reagent Tracking** by choosing reagent A, B, C, D, E or F. This enables reagent lot number and expiration date tracking.



- 3. Select **Back** to return to System Setup.
- 4. Select Reagents.

5. Select Change next to the appropriate reagent.



6. Scan the reagent bottle barcodes (Section 3.2) or manually enter the reagent information in the correct fields.



- 7. Press Save.
- 8. Repeat steps 5-7 for each reagent.

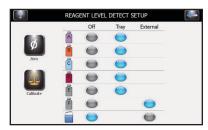
#### **Modifying Level Detect Functions**

The Level Detect function alerts when the reagent is running low, or when the waste container is almost full. Reagent and waste container monitoring can be turned On and Off from the System Setup screen. The system defaults to On (Tray and External) for reagent monitoring and to Off for waste container monitoring. See Section 2.2 for complete instructions.



1. From System Setup, select **Level Detect**.

2. Select the reagent monitoring options to be modified.



- To disable monitoring, select Off next to the appropriate reagents.
- To enable monitoring, select Tray next to the appropriate reagents.
- To monitor reagent F, select External.
- To monitor the waste container, select External.

#### Changing User Language



- 1. From System Setup, press Language.
- 2. Select the software language from the list on the left.

3. Select OK.

#### Setting the Date and Time



- 1. From System Setup, press Set Date/Time.
- 2. Choose 12 for a 12-hour clock or 24 for a 24-hour clock.
- 3. Use the up and down arrows to modify the time and date.
- 4. Press Save.



#### System Log

The instrument records all login, logout, stain or cytocentrifuge cycles, setting changes, maintenance functions and specimen identification (if enabled).

#### Accessing Logs



1. From System Setup, press System Log.

2. Use navigation arrows to scroll through the log.

#### **Exporting Logs**



1. From System Setup, press System Log.

2. Plug a Flash Drive into the right USB port.



3. Press Export.

**NOTE:** The log files are exported as a CSV file to the Flash Drive for access in spreadsheet software programs.

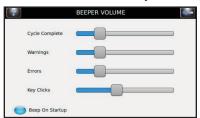


#### **Controlling Beeper Alerts**



1. From System Setup, select Beeper.

2. Use the sliders to modify the beeper volume for Cycle Complete, Warnings, Errors, or Key Clicks.



3. Select Beep On Startup to turn the audible startup alert ON or OFF.

#### QC/Maintenance Tracking

Under system default settings, the following QC/Maintenance Tracking options are disabled:

- · Enable Stain Slide Tracking
- · Enable Cyto Slide Tracking
- Manual Entry
- · Enable Preventive Maintenance Tracking
- · Enable Reagent Tracking

#### Enable Stain Slide Tracking

To activate Stain Slide Tracking:



1. From System Setup, press QC/Maintenance Tracking.

2. Press Enable Stain Slide Tracking.



3. Press **Back** twice to return to the Main screen. Verify that the Start Button on the main screen reads "Load Slides."

NOTE: Selecting Enable Stain Slide Tracking changes the Start button on the Main menu to "Load Slides."



4. Press Load Slides. The Scan and Load Slides menu appears.







- A. If using the barcode reader, scan the specimen slides that contain barcodes. See Scanning Slides with the Barcode Reader (Section 3.2) for complete instructions.
- B. If entering specimen information manually, see Manually Entering Specimen Information (Section 3.2).
- 6. See Section 4 for remaining steps for running a stain cycle.

#### Enable Cyto Slide Tracking

Allows slide tracking in cytocentrifuge mode. See the Cytopro Rotor Applications Manual for complete information.



#### **Enable Manual Entry**

If selected, allows manual entry of slide information using the keypad (limited to 24 characters).



#### **Enable Preventive Maintenance Tracking**



To activate the tracking prompts for Preventive Maintenance Tracking, use the following steps:

- 1. From System Setup, select QC/Maintenance Tracking.
- 2. Select Enable Preventive Maintenance Tracking.



3. Enter the information for the Daily, Weekly, and QC Slide prompts in corresponding fields. See Using the Preventive Maintenance Log (Section 5.1).

#### Enable Reagent Tracking



To activate Reagent Tracking:

- 1. From System Setup, select QC/Maintenance Tracking.
- 2. Select Enable Reagent Tracking.



3. Select the reagent to be tracked (A, B, C, D, E, F).

#### Restoring Software Defaults



1. From System Setup, select Restore Defaults.

#### **⚠** CAUTION:

#### Restoring the system defaults will remove all personal settings.

- · Restoring System Settings will delete all usernames and passwords.
- · Restoring Stain Settings will delete all stain programs and restore the default programs.
- Restoring Cytocentrifuge Settings will delete all cytocentrifuge programs and restore the default programs.
- 2. Select the settings to restore to factory defaults: System Settings, Stain Settings, or Cytocentrifuge Settings.
- 3. Press Restore.



4. The display returns to the Main menu.

### 3.2 Recording Specimen and Reagent Information

#### Scanning Slides with the Barcode Reader



1. From System Setup select QC/Maintenance Tracking.

2. Select Enable Stain Slide Tracking.

**NOTE:** Selecting Enable Stain Slide Tracking changes the Start button on the Main menu to "Load Slides." See Scanning Slides with the Barcode Reader.



- 3. Press **Back** twice to return to the Main menu.
- 4. Press Load Slides on the Main menu. The Scan and Load Slides menu will appear.



- 5. Scan the barcode of each slide in the batch and load into the carousel according to instructions in Section 4.1.
- 6. Verify that each barcode appears on the Scan and Load Slides menu.



7. When preparations to stain have been completed, (Section 4) press Start.

#### Scanning Reagent Bottles with the Barcode Reader



1. From System Setup select QC/Maintenance Tracking.

2. Select Enable Reagent Tracking for each desired reagent (A, B, C, D, E, F).



- 3. Press **Back** to return to the System Setup menu.
- 4. Press **Reagents** to reveal the Reagent Information screen.



5. Press Change next to the desired Reagent (A, B, C, D, E, F).



6. Scan the barcode of each enabled reagent bottle.



- 7. Press Save.
- 8. Verify that the barcode appears on the Reagent Information menu.



9. Repeat steps 4-8 for each reagent bottle that is enabled in QC Maintenance Tracking.

**NOTE:** Reagent Information can be accessed directly by pressing the bottle icons on the right side of the Main menu, then reagent information can be scanned or manually entered by pressing Change next to the desired reagent.

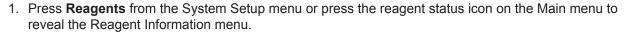
#### Manually Entering Specimen Information

With Stain Slide Tracking and Manual Entry enabled in the QC Maintenance menu:

- 1. Press Load Slides on the Main menu.
- 2. Press Add to reveal the keypad.
- 3. Enter slide information (maximum of 24 characters) and press Enter.
- 4. To change or delete the entry, select the entry on the display and press **Edit** or **Remove**.
- 5. Load slides and run stain cycle as shown in Section 4.1.

#### Manually Entering Reagent Information







- 2. Select the desired reagent and press Change.
- 3. Press the desired field (Reagent REF, Expiration Date, or Lot Number (Serviced Date/Time automatically gets entered); enter the information on the keypad and press **Enter**.



**NOTE:** Reagent REF number must be a valid ELITechGroup REF number for the selected reagent. Incorrect entries will generate an error message.



4. When all the information has been entered, press Save.

### 3.3 The Help Menu

The Help menu is a comprehensive onscreen help function that provides detailed information on the following subjects:

- · Help Screens
- Basic Operation
  - ♦ Loading the Carousel
  - ♦ Programming Number of Slides
  - ♦ Correct Reagents and Locations
  - ♦ Selecting a Staining Program
- · System Setup
  - ♦ Setting up Stain Programs
  - ♦ System Setup Help
  - ♦ Setting up Cyto Programs
  - ♦ Setting up Users
  - ♦ Setting up Level Monitoring System
  - ♦ Setting Instrument Language
  - ♦ Setting the Date and Time
  - ♦ Instrument Logging
  - ♦ Setting Instrument Beeps
  - ♦ Calibrating Touch Screen
  - ♦ Restoring Instrument Defaults
- Maintenance Functions
  - ♦ Pattern Tests
  - ◊ Volume Tests
  - ♦ Line Flush
  - ♦ 60-Second Prime
- Cleaning the Instrument
- Cytocentrifuge Use



### **Using Help**



- 1. Press **Help** to access the help function.
- 2. Select the desired topic.
- 3. Use the direction arrows to navigate.
- 4. Press **Exit** to return to the Main menu.

### 4.1 Operating Instructions

#### Suggested Staining Protocol

**NOTE:** Samples and slides should be prepared and fixed according to recommendations in Appendix E or equivalent.

- Perform a Hub Pattern Test (once per day).
- · Select or verify desired stain program.
- If slide tracking is enabled, scan or enter slide information.
- · Load slides into the carousel. Use blocking slides if needed.
- · Place loaded carousel into the stainer and close the lid.
- · Check reagent and waste levels.
- If slide tracking is not enabled, enter the number of slides on the Main menu.
- · Perform a stain cycle.
- · Unload the carousel.

#### Performing a Hub Pattern Test

Use the Hub Pattern Test to ensure the nozzles are clear of debris and spraying properly.

1. From the Maintenance menu, press **Pattern Test**.



- 2. Hold a sheet of white paper towel near the drive hub, squarely facing the target nozzle.
- 3. Press the corresponding button for the reagent line to be tested.



4. Check the pattern. If incorrect, clean the nozzle orifice with the nozzle brush provided in the Nozzle Maintenance Kit. If this fails to correct the problem, refer to Nozzle Maintenance and Performance (Section 6).

Figure 7: Correct Hub Pattern Test Result



Figure 8: Incorrect Hub Pattern Test Result



**NOTE:** If the Hub Pattern Test result is incorrect, clean the nozzle orifice with the nozzle brush provided in the Nozzle Maintenance Kit or disassemble nozzle and clean all pieces (Section 6).

**NOTE:** If not staining immediately after Hub Pattern Test, it is recommended to run a clean cycle to prevent concentrated reagent from sitting in the lines for extended periods of time.

#### Loading the Carousel

#### **↑** CAUTION:

Never load chipped or cracked slides into the instrument. Slides in poor condition may break during the staining cycle. If a slide breaks in the bowl, see Cleaning Broken Slides (Section 5.4).

#### **↑** CAUTION:

Keep small ferrous metal objects away from the lab bench. These objects can be attracted to the magnets on the bottom of the carousel and cause damage if spun free during instrument operation.

#### **⚠ CAUTION:**

Load slides in balanced pairs. If staining an odd number of slides, use a blank slide to balance the carousel.

**NOTE:** Load the carousel with similar specimens for a similar level of staining. There is no guarantee of staining performance when dissimilar specimens are used.

- 1. Remove the carousel from the bowl and place it on a solid, level surface.
- 2. Remove the carousel lid by pressing the button and lifting the lid.
- 3. If Slide Tracking is enabled, press Load Slides.
  - If using the barcode reader, scan each specimen slide barcode before loading it into the carousel. Slide Tracking must be enabled from the System Setup menu. See Enable Stain Slide Tracking (Section 3.1).
  - If entering slide information manually, follow the instructions in Section 3.2.
- 4. Insert the slides into the carousel with the first slide in position 1.
  - Load slides in balanced pairs (directly opposite one another) to balance the carousel. If staining an odd number of slides, use a blank slide to balance the carousel.
  - ♦ If there are empty slots in the carousel, use blocking slides to prevent overspray (see below).
  - When using a 12-Slide carousel, load slides with the label end to the outside of the carousel.
  - When using a 30-Slide carousel, load slides with the label end towards the hub (center). If the label end is to the outside, the spray does not get all the way to the hub because of the smaller corridor.
  - ♦ Always load slides with the specimen facing clockwise.
  - Always place the first slide in position 1, the second in position 2, and so on.

NOTE: A warning will sound during the staining cycle if the carousel is unbalanced.





Figure 9: Loading a 12-Slide Carousel (Slide Labels Toward Outer Rim)

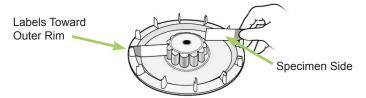


Figure 10: Loading a 30-Slide Carousel (Slide Label Toward Center)

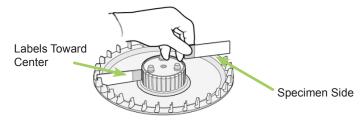
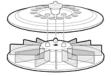


Figure 11: Reattaching the Carousel Lid



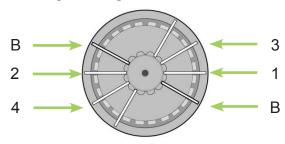
- 5. Reattach the carousel lid by pressing the button and lowering the lid over the indexing posts.
- 6. Release the button and press the lid handle until it is firmly closed and locked.

#### **Using Blocking Slides**

If the carousel is not full, blank slides should be used as blocking slides. Blocking slides prevent overspray of reagents onto the specimen slides. Overspray can cause slides to become over-stained.

• Place a blocking slide in front of position 1 and 2.

Figure 12: Using Blocking Slides



- B Blocking Slide
- 1 Specimen slide in carousel position 1
- 2 Specimen slide in carousel position 2
- 3 Specimen slide in carousel position 3
- 4 Specimen slide in carousel position 4

#### Performing a Stain Cycle

NOTE: Sample slide preparation guidelines can be found in Appendix E.

1. Insert a carousel loaded with specimen slides and close the instrument lid.



2. If Slide Tracking is not enabled, select the number of slides to be stained. Slide selection defaults to full carousel at the end of the run, after pressing Stop, or selecting a number greater than the full carousel default.

**NOTE:** To stain an odd number of specimen slides, select the next higher number listed on the display. For example: to stain 3 slides, select 4. To stain 7 slides, select 8, etc.



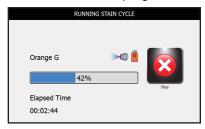
**NOTE:** If entering slide information by barcode reader or keypad, the number of slides is programmed automatically. Adjust the total number of slides if adding other specimen slides that have not been entered by barcode reader or keypad.

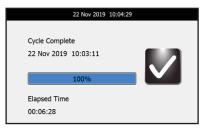
**NOTE:** Do not include blocking slides in the total number of slides.

3. If the desired stain program appears on the display, proceed to Step 4. If the desired program does not appear on the display, press Programs. Then select the desired program and proceed to Step 4.



4. Press **Start**. The display shows the progress of the stain cycle. Upon completion of the stain cycle, displays Cycle Complete and a signal tone sounds (if enabled). Displayed Elapsed Time and progress % varies based on program settings.





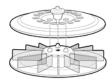
**NOTE:** Use the emergency Stop button when required, for example, if there is abnormal vibration or noise. Pressing Stop aborts the stain cycle.

#### **Unloading the Carousel**

#### **⚠** WARNING!

Treat slides in accordance with good laboratory practices and local regulations.

- 1. Remove the carousel from the bowl and place it on a solid, level surface (carousel may not be dry).
- 2. Remove the carousel lid by pressing the button and lifting the lid.



3. Carefully remove each slide, coverslip, and observe the specimen using a microscope.

#### Monitoring Reagent and Waste Levels

If enabled, the stainer displays the approximate reagent and waste container levels and other information.

#### **⚠ CAUTION:**

Monitor the reagent and waste container levels on the display (if enabled) and by direct inspection of the bottles. The monitor will show the approximate level of each reagent. This can be compared to the actual level in the bottles.

- Never allow a reagent to run dry. When the reagent level is near empty, replace the reagent bottle with a new one (see below).
- Never allow the waste container level to go above the maximum safety level.

Table 10: Reagent Level Detect Display Symbols (Reagent A shown)

Symbol	Description
A	Reagent unselected in Level Detect
A	Reagent Bottle Full
A	Reagent Bottle 2/3 Full
A	Reagent Bottle 1/3 Full
<b>&amp;</b>	Reagent Bottle Empty
!	Measurement Error (such as external bottle unplugged)
	Reagent has exceeded expiration date (enabled from QC Maintenance menu)
	Waste bottle empty
	Waste bottle error
×	Waste bottle full (such as external bottle unplugged)

**NOTE:** Access the Reagent Information menu by pressing the bottle icons on the right side of the Main menu. Press **Change** to scan or manually enter reagent information.

**NOTE:** Do not put residual reagent from a used bottle into a new bottle. This can lead to an accumulation of residue on the slides and may be a source of contamination.

#### ▲ WARNING!

Reagents used in this instrument contain moderately hazardous chemicals that require care in handling. Always use appropriate safety measures, including gloves and eye protection, when handling reagents.

#### Replacing a Reagent Bottle

- 1. Remove the empty reagent bottle from the tray but do not disconnect the dip tube.
- 2. Open the new bottle and record the letter on the cap for future use, such as long-term storage.
- Open the Reagent Information menu by pressing the reagent bottle icon on the right side of the Main menu.
- 4. Select the desired reagent and press Change.
- 5. When using reagent tracking, scan the barcode, or manually enter the reagent REF, expiration date and lot number in the Reagent Information menu (Section 3.1).
- 6. Unscrew the cap and remove the dip tube from the empty bottle.
- 7. Insert the dip tube into the new reagent bottle and screw on the cap.
- 8. Place the new bottle in the tray.

#### Adding Reagent F to the External Bottle

- 1. Open the Reagent Information menu by pressing the bottle 'F' icon on the right side of the Main menu.
- 2. Press Change next to bottle 'F'.
- 3. When using reagent tracking, scan the barcode, or manually enter the REF (SS-051F), expiration date and lot number in the Reagent Information menu (Section 3.1).
- 4. From the external bottle, unscrew the cap and remove the level detect.
- 5. Open the new SS-051F bottle.
- 6. When adding SS-051F with the same lot number to the external bottle, pour the contents from the new SS-051F bottle into the external bottle.
- 7. When adding SS-051F with a different lot number to the external bottle:
  - A. Pour any remaining reagent from the external bottle into the bowl of the stainer.
  - B. Pour approximately 10-15 mL of the new bottle of acid alcohol wash (SS-051F) into the external bottle, swirl the external bottle, and pour the rinse into the bowl of the stainer.
  - C. Pour the remaining contents from the new SS-051F bottle into the external bottle.
- 8. Insert the level detect sensor into the external bottle and tighten the bottle cap.
- 9. Reposition the external bottle.

#### **Emptying the Waste Container**

The Reagent Level Detect function, automatically monitors the waste level and indicates when the waste container should be emptied. It is still necessary to check waste levels visually to ensure the waste container does not overfill.

#### **⚠** CAUTION:

Dispose of collected waste according to local statutes and safety requirements.



- 1. Unscrew the cap from the full waste container.
- 2. Discard the waste according to local regulations.
- 3. Reinstall the cap on the empty waste container.

### 5.1 Preventive Maintenance

The system provides a Preventive Maintenance Log for tracking the most recent maintenance activities. See Enable Preventive Maintenance Tracking (Section 3.1) and Using the Preventive Maintenance Log in this section.

#### Daily Maintenance/Quality Control (QC)

- 1. Check reagent levels and expiration dates.
- 2. Empty the waste container if necessary.
- 3. At the beginning of the day:
  - ♦ Perform a Hub Pattern Test. (Section 4.1) Perform a Slide Pattern Test if a Hub Pattern Test produces a normal result, but staining is still inadequate. (Section 6.6)
  - ♦ Run a QC slide if required by your laboratory.

NOTE: If staining will not be performed immediately, run a clean cycle after Hub Pattern Test.

- 4. If necessary, use the nozzle brush from the Maintenance Kit to clean the nozzle orifices. Press the individual bristles into the nozzle openings.
- 5. At the end of the day, end of each shift, or if the instrument will be idle for more than four hours:
  - Place an empty carousel in the bowl and close the lid. Press Standby/Ready on the front panel. Press the check box confirming that slides are removed from the carousel and wait until the end of the automatic cleaning process, or press Clean.
  - ♦ Spray and wipe the bowl, interior lid, and nozzles with 70 to 100% alcohol or SS-029 nozzle cleaning solution. If necessary, use the nozzle brush from the maintenance kit to clean the nozzle orifices. Press the individual bristles into the nozzle openings.
  - Wipe down the exterior of the instrument with 70 to 100% alcohol or SS-029 nozzle cleaning solution. Wipe clean with a paper towel.
- 6. Ensure the maintenance procedures listed on the Maintenance Log have been performed and entered into the Preventive Maintenance chart or log.

#### Weekly Maintenance

- 1. Perform a Hub Pattern Test (Section 4.1). Perform a Slide Pattern Test when a Hub Pattern Test produces a normal result, but staining is still inadequate. (Section 6.6)
- 2. Perform a Volume Test (Section 6.4).
- 3. Manually clean the nozzles if necessary (Section 6.1). If volume trends lower or spray pattern is abnormal, disassemble and clean affected nozzle(s). Do not mix or interchange nozzles or nozzle parts. Always return nozzles to same position in stainer. Repeat Hub Pattern Test and Volume Test on cleaned nozzle(s). Perform the Slide Pattern Test when a Hub Pattern Test produces a normal result, but staining is still inadequate.

NOTE: If staining will not be performed immediately, run a clean cycle after Volume and Hub Pattern test.

- 4. Wipe down nozzles, carousel tray and carousel lid using 70 to 100% alcohol or SS-029 nozzle cleaning solution. Wipe clean with a paper towel.
- 5. Slowly pour 200-300 mL of water into instrument drain to prevent buildup of paper fibers, precipitates, etc. Verify drain is flowing properly and not allowing fluid to back up in bowl or flow out of air vent on case back.
- 6. Ensure the maintenance procedures listed on the Maintenance Log have been performed and entered into the Preventive Maintenance chart or log.

**NOTE:** If not staining immediately after Hub Pattern Test, it is recommended to run a clean cycle to prevent concentrated reagent from sitting in the lines for extended periods of time.

#### Monthly Maintenance

- 1. Disassemble and manually clean all nozzles. Refer to Nozzle Disassembly and Cleaning (Section 6.1).
- 2. Ensure the maintenance procedures in the Preventive Maintenance Log have been performed and entered. The Preventive Maintenance chart may also be used.



NOTE: If staining will not be performed immediately, run a clean cycle after Volume and Hub Pattern test.

#### Annual Maintenance

- 1. Check internal and exterior tubing and fittings for cracks, leaks, or any type of deterioration. Replace as needed.
- 2. Run a line flush for the Hematoxylin line D as described in Section 6.5.

#### Using the Preventive Maintenance Log

With Preventive Maintenance Tracking enabled, the Preventive Maintenance Log provides a convenient and structured means of recording important maintenance and QC functions. The system allows the setup of timely prompts that require response by the user. See Enable Preventive Maintenance Tracking (Section 3.1).



1. From the Maintenance menu, press **QC/PM** to open the Preventive Maintenance Log.



2. Press Record Maintenance.



Maintenance Task entry options:

QC Slide Staining (Drop Down Menu)

Not Completed Acceptable

. Unacceptable

Inconclusive

Disinfect Reusable Bottles

Completed (Select/Deselect)

Drain Check

Completed (Select/Deselect)
Manual Nozzle Cleaning
Completed (Select/Deselect)

3. Press **Save** to record entries.



**NOTE:** Check interior and exterior tubing and fittings for cracks, leaks, or any type of deterioration. Replace as needed.

NOTE: Run a line flush as described on page 68.

## 5.2 Storing the Instrument

If the instrument will be inactive for more than one week, the recommendation is to perform the long-term storage procedure. This will prevent nozzles from clogging when the instrument is reactivated.

#### Preparing for Long-Term Storage

- 1. With the carousel removed, remove and clean the nozzles. Store nozzle parts in tubes that are labeled to indicate their correct position.
- 2. Unscrew the cap and remove the dip tube from the reagent bottles.
- 3. Place the end of the dip tube in a bottle of 50% DI water/50% methanol.
- 4. Flush at least 250 mL of 50% DI water/50% methanol through each reagent line by priming all lines simultaneously. Leave the 50% DI water/50% methanol in the line.

#### **↑** CAUTION:

Leave 50% DI water/50% methanol in the reagent lines during storage. Allowing reagent lines to run dry can damage the instrument.

#### **⚠** CAUTION:

Do not subject the instrument to freezing temperatures. Freezing of aqueous fluids in the lines may cause damage to the instrument.

- 5. Flush the bowl with 50% DI water/50% methanol.
- 6. Install nozzles stored in tubes in Step 1 to their original positions.

#### Preparing for Operation after Storage

Follow the Setup and Preparation for Operation instructions (Section 2).

### 5.3 Replacing Fuses

#### **⚠** WARNING!

To prevent the risk of fire, the main fuses must only be replaced with fuses of the same type and rating. Recurring fuse failure indicates serious internal problems, if this occurs, contact ELITechGroup.

- 1. Power **OFF** the instrument.
- 2. Disconnect the power cord from the power outlet and the rear panel of the instrument.
- 3. Open the fuse cover by inserting a screwdriver in the slot on the right side of the cover and gently prying the cover out.
- 4. Remove the fuse holders to inspect the fuses.
- 5. Replace the fuses if necessary.
- 6. Push the fuse holders in.
- 7. Close the fuse cover.
- 8. Reconnect the main power cord to the rear panel of the instrument and to the power outlet.
- 9. Power **ON** the instrument.

### 5.4 Cleaning the Stainer and Carousels

#### **⚠** WARNING!

All cleaning procedures should be performed in a well-ventilated room by authorized and trained personnel wearing appropriate protection equipment.

- 1. Clean the outside of the instrument with 70 to 100% ethanol or methanol or SS-029 nozzle cleaning solution.
- 2. Clean the carousel and lid with 70 to 100% ethanol or methanol or SS-029 nozzle cleaning solution.

**NOTE:** Freshly prepared (< 24 hours old) 10% bleach solution can be used as well. The 10% bleach solution helps clean the stained areas.

#### Cleaning Liquid Spills

Remove any liquid spilled on the instrument immediately to avoid damage to the instrument.

#### WARNING!

If potentially infectious liquid is spilled on the instrument, the instrument must be disinfected in accordance with all applicable local regulations. Refer to Decontaminating the Stainer and Carousels (below) for instructions.

#### Cleaning Broken Slides

Take stringent precautions if a slide breaks inside the instrument during a staining cycle, especially if the instrument has been processing dangerous specimens. Always use protective gloves, safety glasses, and forceps when removing broken glass from inside the instrument.

- Glass shards embedded in the walls of the bowl can cause serious cuts and pose a risk of infection.
- · Always remove embedded shards with a scraper before attempting to remove loose glass.
- Use a vacuum or adhesive tape to pick up loose glass inside the stainer bowl.

#### Cleaning External Bottle

- 1. Unscrew the cap and remove the level detect from the external bottle containing the acid alcohol wash.
- 2. Pour any remaining reagent from the external bottle into the bowl of the stainer.
- 3. Open a new SS-051F bottle.
- 4. Pour approximately 10-15 ml of the new bottle of acid alcohol wash (SS-051F) into the external bottle, swirl the external bottle, and pour the rinse into the bowl of the stainer.
- 5. Reposition the cap on the new SS-051F bottle.

### 5.5 Decontaminating the Stainer and Carousels

All parts of the instrument that come into contact with biological specimens, patient specimens, positive control specimens, or hazardous material must be treated as potentially infectious.

Before the instrument is returned for service, all outer surfaces must be decontaminated. The operating authority must complete a disinfection declaration, otherwise the instrument may be rejected by the distributor or service center or quarantined by customs authorities.

#### **⚠** WARNING!

Reagents used with the instrument contain moderately hazardous chemicals that require care in handling. Always use appropriate safety measures including gloves and eye protection, when handling reagents.

#### WARNING!

Authorized and trained personnel wearing appropriate protection equipment should perform the decontamination procedure in a well-ventilated room. It is very important to thoroughly decontaminate the instrument before removing it from the laboratory or before performing any technical service. This procedure may not be effective against prions.

#### **⚠** WARNING!

Prior to decontaminating, disconnect the instrument from the main power supply to avoid any risk of fire and explosion.

#### ▲ WARNING!

The decontamination procedure and the disinfectants must comply with the local applicable regulations.

#### Solutions for Decontaminating the Instrument

The outer surfaces of the instrument should be decontaminated using a decontaminating solution such as:

- 70% ethanol or methanol
- · Mild detergent
- 10% bleach solution (< 24 hours old)</li>
- Decontamination Solution (REF: SS-133)

Figure 13: Lid Latch and Locking Pin Hole Locations



- 1. Lid Latch Hole
- 2. Locking Pin Hole

#### Decontaminating the Instrument

- 1. Prepare a suitable container for all disposables.
- 2. Cover the lid latch and locking-pin holes with waterproof tape to protect the interior (Figure 13).
- 3. Place the instrument in a biological safety hood or well-ventilated area.
- 4. Spray the inner bowl and inner lid with a decontaminating solution such as Ref: SS-133.

- 5. Repeat the spray treatment every 2 or 3 minutes for a total of 20 minutes. Do not allow cleaning solutions to dry on the instrument surfaces.
- 6. Rinse the inner bowl and lid thoroughly with water.
- 7. Spray and wipe the exterior surfaces with decontamination solution such as REF: SS-133.

#### **A** CAUTION:

Do not flood the display panel with excessive moisture. Any moisture that seeps through could damage the internal electronics.

- 8. Repeat the spray treatment of exterior surfaces every 2 or 3 minutes for a total of 20 minutes. Do not allow cleaning/decontamination solutions to dry on the instrument surfaces.
- 9. Wipe surfaces thoroughly with a cloth soaked in water until all decontamination solution has been removed.
- 10. Immerse or generously spray the carousel and lid with decontaminating solution. Allow the solution to react for 20 minutes.
- 11. Thoroughly rinse the carousel and lid with deionized or distilled water.

### 5.6 Shipping or Disposing of the Stainer or Carousels

#### Shipping the Instrument or Carousels

#### **⚠** WARNING!

Disinfect the instrument or carousels before returning them to ELITechGroup. The operator must complete a Hazard Free Certification form, otherwise the distributor or service center may not accept the instrument; or customs authorities may hold it.

#### **⚠** WARNING!

Shipping the instrument or carousels without decontaminating according to these instructions is dangerous to service personnel. Additional fees for decontamination performed by ELITechGroup will be charged to the user.

#### **↑** CAUTION:

Ship the instrument or carousels in containers comparable to the original packaging.

#### Hazard Free Certification

The operator must print and complete the Hazard Free Certification (obtained from ELITechGroup Customer Service).

Attach the certification form to the top of the instrument package before sending the package to ELITechGroup.

#### Disposing of the Instrument or Carousels



The instrument and carousels should be completely decontaminated and disposed of as follows:

Under Directive 2012/19/EU (WEEE), this equipment cannot be disposed of in a municipal waste. Instead, the equipment must be disposed of either by:

1. Routing to an authorized local facility approved for handling hazardous materials.

OR

2. Returning the equipment to ELITechGroup or an authorized service center.

### 6.1 Nozzle Disassembly and Cleaning

Nozzle maintenance requires the nozzle maintenance kit and SS-029 nozzle cleaning solution.

#### **⚠** WARNING!

Always wear protective clothing and eye protection when using SS-029 nozzle cleaning solution. Dispose of used solution properly.

**NOTE:** If the compression screw cannot be easily loosened, use light penetrating oil and a 5/8-in. wrench to loosen the nozzle.

**NOTE:** Do not mix or interchange nozzles or nozzle parts. Always return nozzles to the same position in the stainer

#### Nozzle Disassembly

1. Remove the nozzle using the nozzle tool from the nozzle maintenance kit.



2. Disassemble the nozzle. See Figure 5: Nozzle Diagram in Section 1.



**NOTE:** If the compression screw cannot be easily loosened, use light penetrating oil and a 5/8-in. wrench to loosen the nozzle.

3. Place the nozzle parts in a 50 mL conical tube that has been clearly marked with the correct nozzle position.



4. Repeat Steps 1 through 3 for each nozzle.

#### **Nozzle Cleaning**

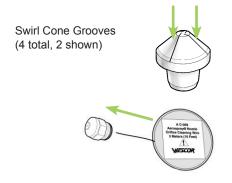


- 1. Fill each 50 mL tube with 25 mL of prepared SS-029 nozzle cleaning solution and cap the tube.
- 2. Gently invert the tube at least ten times to ensure all parts come in contact with the cleaning solution.
- 3. Place the tube in the correctly marked position in the provided tube stand. Soak the parts as long as possible.

**NOTE:** Soak nozzle parts for at least 15 minutes. Parts can be soaked in Nozzle Cleaning Solution overnight.

4. Repeat steps 1 through 3 for each nozzle.

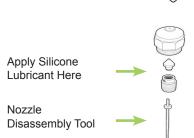
### 6.2 Nozzle Reassembly



- 1. Hold your thumb or a nozzle strainer over the end of the tube to keep the nozzle parts in the tube. Discard the cleaning solution according to applicable statutes.
- 2. Inspect the nozzle parts. Remove any material in the swirl cone grooves by sliding a piece of paper along each of the 4 grooves.
- 3. Thread the nozzle orifice cleaning wire (REF: AC-059) through the back of the disassembled nozzle housing.



- 4. Place the nozzle parts back into the tube and rinse them with water.
- 5. Rinse the parts again with alcohol.



- 6. Apply a small amount of silicone lubricant to the compression screw threads.
- 7. Reassemble the nozzle by placing the compression screw on the nozzle disassembly tool, then inserting the swirl cone into the compression screw.

**NOTE:** Hold all the parts in a vertical position during reassembly.



- 8. Reinstall the nozzle housing over the swirl cone and compression screw.
- 9. Reinstall the nozzle insert.



- 10. Return the assembled nozzle to its original position in the instrument.
- 11. Repeat Steps 1 through 10 for each nozzle.
- 12. Perform a Hub Pattern Test (Section 4).
- 13. Perform a Volume Test (Section 6.4).

**NOTE:** You must perform the Hub Pattern Test and Volume Test before operating the instrument. If the results are incorrect, manually prime the instrument. If not staining immediately after Hub Pattern Test, it is recommended to run a clean cycle to prevent concentrated reagent from sitting in the lines for extended periods of time.

### 6.3 Manual Priming











- 1. Remove the carousel from the bowl.
- 2. Remove the nozzle connected to the line to be manually primed.
- 3. Insert the priming tool nozzle adapter (included in the Nozzle Maintenance Kit) into the nozzle holder.
- 4. Turn it clockwise to install the adapter into the holder.
- 5. Withdraw the priming tool plunger halfway to create a vacuum. Hold the plunger in position.

- 6. Press Maintenance from the Main menu.
- 7. Press Volume Test.
- 8. Press the desired prime button to start the reagent pump.
- 9. Run the reagent into the tube until the fluid is free of bubbles, then press **Stop**.

#### ♠ WARNING!

Do not pull the plunger completely out of the priming tool. Pulling the plunger out of the tool may result in splashing or spraying of reagents. Do not push the plunger in while it is connected to the nozzle holder.

- 10. Turn the nozzle adapter counterclockwise to remove it from the nozzle holder.
- 11. Discard the collected fluid into the stainer bowl.
- 12. Reinstall the nozzle.
- 13. Perform the Hub Pattern Test and record results.
- 14. Perform the Volume Test and record results.

**NOTE:** If not staining immediately after Hub Pattern Test, it is recommended to run a clean cycle to prevent concentrated reagent from sitting in the lines for extended periods of time.

### 6.4 Performing the Volume Test

The Volume Test requires the Nozzle Maintenance Kit.

**NOTE:** The Volume Test must be performed weekly.







- 1. From the Maintenance menu, select **Volume Test.**
- 2. Hold a Volume Test tube (small tube) to cover the selected nozzle.
- 3. Press the corresponding reagent button to collect the reagent.

**NOTE:** With QC/Maintenance tracking enabled, enter the measured volume on the keypad and press ENTER. With QC/Maintenance Tracking disabled, the menu returns to the Maintenance menu.

- 4. Remove and cap the tube.
- 5. Record the nozzle position on the tube.
- 6. Place the tube in the appropriate position in the tube stand.
- 7. Repeat Steps 2 through 6 for each nozzle.
- 8. Compare collected nozzle volumes with the following table.

Table 11: Volume Test Tolerances

Nozzle/Reagent Line	Minimum	Maximum
А	9.0 mL	11.0 mL
В	9.0 mL	11.0 mL
С	9.0 mL	11.0 mL
D	7.5 mL	10.0 mL
E	9.0 mL	11.0 mL
F	9.0 mL	11.0 mL

**NOTE:** The stainer normally functions correctly if nozzle volumes are slightly higher or lower than the specified range. Spray volumes < 7.0 mL or > 11.0 mL indicate serious problems with the nozzles or reagent delivery lines.

- If the volume is within the tolerance range, go to Step 9.
- If the volume is outside the tolerance range:
  - A. Clear the nozzle orifice with the nozzle brush found in the maintenance kit. Press the individual bristles into the nozzle openings.



- B. If necessary, remove the nozzle and perform the Nozzle Cleaning procedure (Section 6.1).
- C. If the problem persists, replace the nozzle.

**NOTE:** If the problem persists after replacing the nozzle, contact ELITechGroup.

- 9. Prepare the Maintenance Kit for future use:
  - ♦ Empty the contents of the tubes into the stainer bowl.
  - ♦ Rinse the tubes with water.
  - ♦ Place the tubes back into their original place in the Maintenance Kit or tube stand.
- 10. Press **Back** twice to return to the Main menu.

#### 6.5 Line Flush

The Line Flush is a semi-automated procedure for cleaning the reagent lines. The Line Flush allows flushing the D line, the A and B lines, or all three lines at the same time. Follow the screen prompts as the sequence progresses. Once the process is started and sequences past the first step, the remaining Line Flush sequence must be completed before continuing with normal staining operations.

Recommendation is to perform the Line Flush procedure annually to help prevent nozzle plugging or when troubleshooting a staining issue. This procedure requires the nozzle maintenance kit.

**NOTE:** The Line Flush procedure requires a minimum of 1 hour and at least 300 mL of SS-029 (diluted SS-029C) nozzle cleaning solution.

**NOTE:** A carousel must be in place during the procedure or the instrument will generate an error and abort the procedure.

From the Maintenance menu, press Line Flush.

#### Line Flush Step 1

- 1. Select the desired line or lines to be flushed (D, A and B, or both).
- 2. Remove the DF, ABCE nozzle, or both, depending upon the desired line flush.
- 3. Load 500 mL of DI water for each line(s) that is being flushed.
- 4. Insert an empty carousel and close the lid.
- 5. Press **Start**. The instrument pumps approximately 400 mL of water through the line(s). A status bar indicates progress and automatically advances to the next step.

#### Line Flush Step 2

- 1. Remove the remaining DI water.
- 2. Load at least 300 mL of the prepared cleaning solution (diluted SS-029C) for each line(s) that is being flushed.
- 3. Press **Continue**. The instrument pumps approximately 200 mL of the prepared cleaning solution through the line(s) and then starts a 60-minute countdown timer.
- 4. After the timer completes, automatically advances to the next step (the instrument may remain idle up to a maximum of 12 hours).

#### Line Flush Step 3

- 1. Remove the remaining cleaning solution.
- 2. Load 500 mL of DI water for each line(s) that is being flushed.
- 3. Press **Continue**. The instrument pumps approximately 400 mL of water through the line(s). A status bar indicates progress and automatically advances to the next step.

#### Line Flush Step 4

- 1. Remove the remaining DI water.
- 2. Load at least 300 mL of the correct reagent for each position.
- 3. Press **Continue**. The instrument pumps approximately 200 mL of the reagent(s) through the line(s) and automatically advances to the next step.

#### Line Flush Step 5

- 1. Reinstall the nozzle(s).
- 2. Press Continue. The stainer primes the line(s) and returns to the Main menu.

**NOTE:** If not staining immediately after Hub Pattern Test, it is recommended to run a clean cycle to prevent concentrated reagent from sitting in the lines for extended periods of time

### 6.6 Performing the Slide Pattern Test

This test can differentiate poor staining results from sample preparation problems, or nozzle obstructions. Perform the Slide Pattern Test when a Hub Pattern Test produces a normal result, but staining is still inadequate.

- 1. Place a 1 x 3 inch (2.5 x 7.6 cm) piece of paper (REF: RP-500) in positions 1 and 2 of the carousel, with a blocking slide in front of positions 1 and 2.
- 2. Load the carousel into the stainer and close the lid.



3. From the Main menu, press Maintenance.



4. Press Pattern Test.

5. Press the corresponding button for the reagent line to be tested.



- 6. Remove the paper slides.
- 7. Repeat Steps 1 through 6 for each reagent line.
- 8. Examine the paper slides for each reagent. The pattern on the slide should be uniform, without any continuous lines or streaks (colors vary based on reagent).

Figure 14: Correct Slide Pattern Test Result

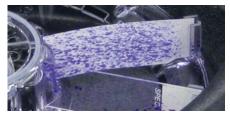
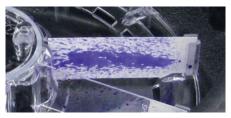


Figure 15: Incorrect Slide Pattern Test Result



9. If the result is incorrect, clear the nozzle blockage using the nozzle brush, or disassemble and clean the nozzle (Section 6.1).

NOTE: If not staining immediately run a clean cycle after Volume an Hub Pattern tests.

## **Section 7: Solving Problems**

## 7.1 Troubleshooting

The following table is to help identify and solve routine problems with the stainer. More difficult problems may require technical service. Contact your ELITechGroup representative for assistance.

#### **⚠** WARNING!

Due to the electrical shock hazard, do not open this instrument or attempt internal repairs. Refer servicing to qualified service personnel. Contact your dealer or ELITechGroup Service.

Table 12: General Troubleshooting and Diagnosis

Problem	Solution
There is no power to the stainer when the power switch is turned On.	Check the facility outlet and the power cord connection.
	Check the fuses. Refer to the Replacing Fuses procedure.
	<b>△CAUTION:</b>
	Fuse failure may indicate a serious internal problem.
Strange information shows on the display, and/or erratic stainer operation.	Switch the power <b>OFF</b> , wait 10 to 20 seconds, then switch power <b>ON</b> again. If problem recurs, install a computertype surge suppressor to protect the instrument from power line transients. If possible, connect the stainer to a power circuit that is not shared by centrifuges, refrigerators, air conditioners, or other motorized equipment.  If the above steps do not solve the problem, consult the Aerospray Service Manual, or contact your dealer or ELITechGroup for assistance.
A reagent line will not prime when power is On and you press the prime button.	Follow the procedures in Section 6.3 for priming reagent pumps.
A reagent line will not prime, even with the priming tool (Section 6.3).	Press the priming button and listen carefully for the sound of the pump. If you can hear the pump, try the priming tool again. If the problem is not solved or if you cannot hear the pump there may be an internal problem. Contact your dealer or ELITechGroup for assistance.
Stainer bowl fills with reagent after use.	A small puddle of stain around the drain inlet or the bottom of the bowl is normal. If the bowl is filling with a large quantity of stain, check the external drain tube for blockage. Make sure the drain tube is properly connected and running continuously down toward the lab drain or vented waste container, with no loops, rises, or obstructions. Make sure the end of the tube is not submerged. This can prevent proper drainage. The internal drain may need to be cleaned or replaced. See the Aerospray Service Manual or contact your dealer or ELITechGroup Service.
Stain is leaking onto the counter.	Check all external reagent lines for visible signs of cracks or loose fittings.
	Make sure the drain outlet is not blocked.
	Make sure the drain tube is securely attached to the drain port and that the tubing is not cracked or deformed.
	Reagent leaks may indicate an internal problem (see Section 7.3). See the Aerospray Service Manual or contact your dealer or ELITechGroup for further assistance.

# Section 7: Solving Problems

Problem	Solution
Error messages on the screen.	If the display shows Lid Not Shut: Verify that the lid is fully closed and latched. If the Lid Not Shut indication remains, contact ELITechGroup for assistance.
Wrong Rotor ERROR: 0002	If the display shows Wrong Rotor after pressing Start: Make sure the slide carousel is properly loaded on the drive hub. In staining mode, the instrument detects whether the staining carousel is present before proceeding. In cytocentrifuge mode, the instrument will stop if it senses the staining carousel. After verifying the carousel is correctly loaded, press <b>Start</b> . If the display still shows Wrong Rotor, there may be an internal problem. Check for missing carousel magnets.
	The microprocessor monitors carousel rotation during a staining cycle. The display shows an error message if the rotation is not within the specified range.
Motor Drive Error ERROR: 0008	If the display shows Motor Drive Error: Check the stainer bowl for interference: Turn the hub or carousel by hand; it should turn freely.
	Drive motor or electronic component malfunctions require servicing of internal components. Contact your dealer or ELITechGroup for assistance.
Rotor Imbalance ERROR: 0001	If the display shows Rotor Imbalance, make certain the Cytopro rotor is balanced, or the staining carousel is seated correctly on the hub.
ERROR: 0001	See Electronic Failure later in this table.
The stainer fails to spray reagent during a staining cycle and/or continues to run after the cycle should be complete.	To allow programmed staining of partial loads, the stainer monitors the position of the carousel as it rotates in the bowl. In normal operation, stain is sprayed only in the correct position. This causes the actual cycle time to vary, depending on the position of the carousel at the beginning of the cycle. However, if the cycle continues for an abnormally long period, or if the bar graph and percentage complete icon do not change after 1 minute, it may indicate an electronic problem or an internal problem. To determine this, press <b>Stop</b> .
	If the cycle stops: this indicates a problem with the carousel position sensor. Consult the Aerospray Service Manual or contact your dealer or ELITechGroup for assistance.
	If the cycle continues: this indicates an electronic problem (see below).

# Section 7: Solving Problems

Problem	Solution
Abnormal staining on entire surface of all slides.	Check the reagent level on the display and/ or in the reagent bottles.
	Make sure the external reagent dip tubes are securely attached to each bottle (Section 2.1).
	Open the lid and verify that each reagent pump is primed, by pressing the corresponding prime button. The nozzle should immediately spray a fine mist of reagent. There should be no sputtering or hissing sounds indicating air in the reagent lines.
	Watch the external tubes for air bubbles. Air bubbles indicate inadequate priming or possibly an air or reagent leak in the system. Air in any reagent line will cause poor staining. Refer to Section 6.6 for more information.
	Check nozzle performance using the Slide Pattern (Section 6.6) and Volume Tests (Section 6.4). If necessary, clean nozzle(s) using the procedures in Section 6.1.
	Verify that the vent hole in each reagent dip tube bottle adapter is free of obstructions (this small vent hole is found in the bottle adapter of each reagent line).
	When staining a full carousel (9 or more slides for the 12-Slide carousel or 17 or more for the 30-Slide carousel), make certain the stainer was not programmed for fewer slides.
	If staining a partial load, load the slides in the correct positions as indicated by the markings on the carousel (see Section 4.1).
	Review sample and slide preparation procedures in Appendix E.
Abnormal staining on entire surface of some slides, while other slides from the same carousel appear normal.	Make certain that all position magnets are still attached to the bottom of the carousel. Make certain the stainer was not programmed for fewer slides than was loaded.
	If the stainer is programmed for a partial load, load the slides in the correct positions as indicated by the markings on the carousel (see Section 4.1).
	Verify that each reagent pump is primed by opening the lid and pressing the corresponding prime button. The nozzle should immediately spray a fine mist of reagent. There should be no sputtering or hissing sounds to indicate the presence of air in the reagent lines (see Section 7.3).
	Review sample and slide preparation procedures in Appendix E.
Streaks or bands of discoloration on one or more slides.	Check nozzle spray pattern according to the procedures in Sections 4.1 and 6.6. This type of discoloration is usually caused by debris or reagent precipitate clogging the spray nozzle orifice.
	Clean any nozzle that exhibits a poor spray pattern.
Cells are washing off slides.	Do not use pre-wash option. Use poly-L-lysine (SS-118) coated slides.
	Review sample and slide preparation procedures in Appendix E.
	Call ELITechGroup for information on slide quality.

Problem	Solution
The slides are too red.	An empty Reagent E (alcohol wash) reservoir prevents slides from rinsing well, leaving residual Reagent A (EA-50) on the slide. Check Reagent E level, and if necessary, run the Spray Pattern test to make sure there is proper reagent delivery. See Section 6.6
	Check drain tube for improper installation, or for any blockage in the tubing (such as stain build-up) This can cause staining reagents to accumulate in the bowl leading to incomplete washing of slides. See Section 2.1
	Overall, or predominantly red staining can also occur if the cytoplasmic stain setting is too low. Because the fast-green molecules of Reagent A (EA-50) are larger than the eosin y molecules, they penetrate the cell membrane and stain more slowly, allowing the cells to stain red from the eosin y. To remedy this problem, use a higher cytoplasmic setting.
	Check nozzle delivery and spray patterns before staining and whenever staining problems occur. (See Section 6.6). Inadequate reagent delivery is a primary cause of poor staining.
Cells are too orange.	When smears or cytocentrifuge preparations are not fixed properly, the cytoplasm hardens and shrinks, eventually becoming impermeable to the EA-50 staining. The cells stain orange because the orange G molecule is considerably smaller than the eosin y or fast green molecules and can therefore permeate the dense cell membranes more easily and quickly.
	If a specimen has not been fixed, and then air dries. Soaking the slide in 50% glycerol for 1 hour may rejuvenate some of the cells. If the glycerol does not help, a new smear will have to be prepared.
	Orange staining can also be due to incomplete carbowax removal. Use the pre-wash option or presoak the slides in alcohol before loading them into the stainer. Check to see if the Reagent A (EA-50) nozzle is plugged.
	Check reagent delivery system as outlined in Sections 2, 4, and 6.
Faint staining on top 1/16 inch of slide.	Use the double staining procedure described in Section 13.3
Light, uneven, or no staining.	If staining is even, but too light, increase nuclear or cytoplasmic stain intensity.
	If the staining is uneven, check all nozzles (see Preventive Maintenance chart.) A plugged nozzle may deliver stain, but application will be sporadic and uneven.
	Check for incomplete carbowax removal. Commercially available spray fixatives have varying amounts of polyethylene glycol (carbowax). Carbowax protects cells from air drying during fixation, but if not completely removed can inhibit staining. To remove the polyethylene glycol, soak slides in 95% alcohol or 50% glycerol for 10 to 30 minutes before staining.
	Check to see if slides were placed in the carousel backward or in the wrong position (if staining less than 7 slides). See Section 4.1.

Problem	Solution
Slide is covered with hematoxylin precipitate and nucleus is red.	Check the Reagent C (bluing) reservoir level or check nozzle ABCE for plugging. If bluing is not delivered in sufficient quantities, proper bluing of nucleus and removal of hematoxylin is not possible. Therefore, when the alcohol is atomized onto the slide the hematoxylin precipitates. Since the alcohol has no buffering capacity, the nucleus does not blue properly, and the nucleus remains red.
Nuclear and cytoplasmic staining is not sharp or crisp.	Good nuclear and cytoplasmic staining requires proper fixation and the complete removal of all water from the cells (clearing) prior to coverslipping. Alcohols tend to absorb water from the atmosphere. If cells are not sharp and crisp, replace the Reagent E (alcohol wash) with a fresh supply.
	Then flush the line with 100 mL of fresh reagent. If this does not work, place slides in fresh anhydrous alcohol (or reagent E alcohol wash), followed by xylene or xylene substitute (1 minute each) prior to coverslipping.
	Xylene replaces the alcohol (which absorbs water) with a medium that does not.
Cloudiness appears upon coverslipping.	Xylene and xylene substitutes are not miscible with water, excess water on the slide produces a milky haze over the slide. Endspin can be increased to reduce some cloudiness due to excess water. Replace Reagent E (alcohol wash) with fresh stock and flush line with at least 100 mL. Remove coverslip and mounting media from the slide using xylene or xylene substitute and dehydrate in 100% ethanol, then recoverslip. In areas of high humidity, dip slides in Reagent E for one minute, then xylene or xylene substitute for one minute, then coverslip. In high humidity environments xylene preforms better than xylene substitutes.
Cornflaking artifacts.	Never allow slide surfaces to dry at any time during the staining procedure, including the time prior to coverslipping. If slides are not quickly coverslipped or kept immersed in Reagent E or xylene, cornflaking artifacts may appear. If cornflaking obscures nuclear detail, remove as follows; soak slide in xylene or xylene substitute to remove coverslip. Soak uncoverslipped slide in xylene for 10 to 60 minutes to remove mounting media, then in Reagent E (alcohol wash) for 10 to 60 minutes or until cornflaking disappears. Coverslip the slide.
Microscopic bubbles appear after coverslipping.	To avoid microscopic air bubbles in mounting media, do not use mountant for 16 hours after refilling decanter.
Cell loss.	The following conditions will enhance cell adhesion:
	Use treated slides such as poly-L-lysine. Use spray fixation in place of fixation by immersion in 95% alcohol. Use lower stain intensity settings. Use fresh, unfixed, room-temperature body fluids for cytocentrifugation. Use no Pre-Wash option.
High levels of precipitates observed.	Discard leftover stain in the used bottle when changing to a fresh bottle of stain. While transferring remaining stain saves stain, it eventually concentrates the precipitates to the point they appear on the slide.

Problem	Solution
Electronic Failure	An electronic failure would appear as an obvious malfunction such as a scrambled or totally inoperative display panel.
	Transient voltages coming through the power lines may cause the stainer to "lose its place."
	If this occurs, switch the main power <b>OFF</b> for 10-20 seconds and then back <b>ON</b> to reset the instrument.
	If the problem recurs, install a computer-type surge protector to isolate the instrument.
	If possible, connect the stainer to a power circuit not shared by centrifuges, refrigerators, air conditioners, or other motorized equipment.
	For more obscure electronic problems, monitor the stainer through a complete staining cycle to determine if the operating sequence is correct. Do this by running the stainer while watching the display and listening to the pumps.
	Ensure that each event occurs according to the operating sequence, shown in Table 13 in Appendix D.
	If the problem recurs, contact your dealer or ELITechGroup for assistance.

### 7.2 Instrument Malfunction

#### Air or Reagent Leaks

Repriming the instrument is usually unnecessary unless a reagent bottle runs completely dry.

An air leak is usually to blame if a smooth and continuous liquid spray fails to come from the nozzles. Carefully inspect all components in the external reagent delivery lines. Look for loose connections, cracks, or breaks that might allow air to be drawn in when the pump operates. Replace any defective part or assembly.

Reagent Delivery Lines



An internal leak may cause fluid to leak from the line when the pump is not running. If an abnormal liquid spray still occurs after all the external reagent delivery line components have been verified, the instrument may require service.

A reagent line leak between the pump outlet and the nozzle will cause fluid to leak into the interior of the stainer housing and ultimately onto the counter. If this occurs, the instrument will require service. Contact your dealer or ELITechGroup for assistance.

#### ♠ WARNING!

A break or malfunction in the reagent delivery system can potentially release up to 1000 mL of highly flammable anhydrous alcohol in and around the instrument. If this occurs, carefully shut off the power to the instrument and consult the SDS for information in handling alcohol spills. Do not use the instrument again until any leaks are repaired.

#### ▲ WARNING!

Electrical shock hazard – do not open this instrument or attempt internal repairs. Refer servicing to qualified service personnel. Contact your dealer or ELITechGroup Service.

#### Reagent Level Detect System Errors

#### Reagent A-E Not Calibrated

During the second part of calibration, if no bottles are detected, the display shows an error message.



Calibrate again, making sure that the reagent bottles are inserted in those tray positions that have been enabled in the level detection system.

#### LD (Level Detection) Unstable

If movement was detected on bottles while calibrating/zeroing, the display shows an error message.

**NOTE:** While zeroing or calibrating, do not bump the instrument or lab bench. Ensure that no nearby equipment vibrations can be transmitted to the stainer.

#### Calibrating the Reagent Level Detect System

If the Reagent Level Detect System is reporting incorrectly and zeroing (Section 2.2) does not correct the problem, calibrate the system as follows:



1. Press **System Information** from the Main menu.



2. Press System Setup.



3. Press Level Detect.





4. Press Calibrate. Follow the display prompts.



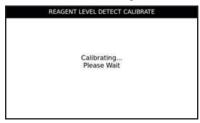
5. Remove all reagent bottles and press Start.



**NOTE:** Any vibrations or bumps to the instrument or lab bench can cause inaccuracies in zeroing or calibration.

**NOTE:** Calibration requires full, unopened (caps and seals in place) 500 mL bottles of reagent, placed in the correct tray positions (due to different densities of each reagent type).

6. Place the correct reagent bottles in all enabled positions, and press Start.



NOTE: The calibration function ignores any disabled reagent line.



- 7. Press **OK**. Press **Back** twice to return to the Main menu.
- 8. Return the reagent bottles to the tray as indicated in Section 2.1 to prepare for staining.

**NOTE:** For accurate reagent level detection and calibration, dip tubes must follow their pre-formed coiled shapes.

#### Calibrating the Touchscreen



- 1. Select and hold **Standby/Ready** for 5 seconds. A calibration screen with a target appears.
- 2. Select the center of the target with a finger, stylus, or similar tool. Another target will appear in a different location.
- 3. Continue to press the center of the targets until all the targets have been pressed (five total). After the fifth target is pressed, the instrument will save the touch screen calibration and return to the Main menu.

### 7.3 Service Information

ELITechGroup's Service Department will help resolve any questions about the operation or performance of the Aerospray Cytology Stainer/Cytocentrifuge.

Customers in the United States should contact us by telephone. Outside the U.S., our authorized dealers offer full local service and support.



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## Section 8: Cytopro® Cytocentrifuge

## 8.1 Cytopro Cytocentrifuge Information

#### **Functional Description**

The Cytopro Cytocentrifuge rotor allows rapid sedimentation of specimen cells onto microscope slides for staining or other purposes. Up to eight disposable/reusable sample chamber assemblies with absorbent pads and glass microscope slides can be loaded into the Cytocentrifuge rotor.

Cytocentrifuge and staining functions are independent of one another.

The Cytopro rotor reduces cell loss during collection and prevents accidental damage to the collected specimen. The rotor is sealed to control aerosol release during cytocentrifugation. See the Cytopro Rotor Applications Manual (Aerospray Models 7xx2) (RP-517) for complete information.

#### Key Features

Adding the Cytopro Cytocentrifuge rotor transforms the stainer into a standard cytocentrifuge with:

- Single, Dual, and Cytopro Magnum chambers
- Reusable or disposable chambers (single and dual)
- · Holds eight slides and chambers
- · User-programmable memory locations for settings (speed, acceleration rate, and time)
- · Easy switching between staining and cytocentrifuge modes
- · Autoclavable rotor

NOTE: Pressing Cyto brings up the Cytocentrifuge mode. Pressing Back returns to stain mode.

#### **⚠** WARNING!

The Cytopro rotor lid, rotor gaskets and related components are intended to be part of biosafety system as specified in international and national biosafety guidelines. They cannot be relied on as the only means of safeguarding workers and the environment when handling pathogenic microorganisms/specimens.

#### Intended Use

The Cytopro Cytocentrifuge rotor is an in vitro diagnostic medical device for professional use only. It is an accessory for fixing biological cell suspensions on glass microscope slides for cytological examination.

The Cytocentrifuge rotor can be used with the following cell suspensions:

- Bronchoalveolar liquid (BAL)
- Cerebrospinal fluid (CSF)
- Urine
- Synovial fluid
- Others

## Appendix A: Reagents (Stains, Washes, Agents)

#### Aerospray® Cytology Reagents

ELITechGroup Aerospray® Cytology reagents are proprietary Papanicolaou staining reagents intended for use in the Aerospray® Cytology stainer in cytology and histology labs.

#### Hematoxylin Stain

ELITechGroup offers Hematoxylin I (SS-051D) and II (SS-051D2). Both hematoxylin stains can be used for either progressive or regressive staining functions. They are suitable for use with all body fluid types intended to be stained with the Cytology stainer. Choice of Hematoxylin and settings would be based on individual laboratory preferences.

#### **Bluing Agent**

The Cytology bluing agent (SS-051C) is a low molarity basic solution which raises the pH of the cells, causing the nucleus to turn blue. While there is no water rinse after the bluing step, no precipitate is formed due to the low concentration of this basic solution.

#### Orange G Stain

The Orange G stain solution (SS-051B) is specifically formulated for staining with the pre-programmed staining times of the Cytology stainer.

#### EA-50 Stain

EA-50 is a counter stain used for cytoplasmic staining (SS-051A). It is specifically formulated for staining with the pre-programmed staining times of the Cytology stainer.

#### Alcohol Wash

The Cytology stainer utilizes a proprietary blend of alcohols (SS-051E) to dehydrate and clear the cells.

#### Acid Alcohol Wash

The Cytology stainer utilizes a proprietary formula (SS-051F) which allows for more control of Hematoxylin stain intensity. Typically, acid-alcohol is used when applying a regressive stain methodology.

#### ▲ WARNING!

To avoid serious instrument damage, never use reagents containing organic solvents unless supplied by ELITechGroup or specified in official ELITechGroup formulation instructions.

See Appendix C for ordering information.

# Appendix A: Reagents (Stains, Washes, Agents)

## **Critical Reagent Components**

Reagent(s)	Critical Components
SS-029 Nozzle Cleaning Solution contains:	40-55% Methyl Alcohol
	1-3% Oxalic Acid
SS-029C Nozzle Cleaning Solution	95-99% Deionized Water
Concentrate contains:	1-5% Oxalic Acid
SS-051A Aerospray® Cytology EA-50 Stain	70-80% Ethanol
SS-051B Aerospray® Cytology Orange G Stain	80-90% Ethanol
SS-051C Aerospray® Cytology Bluing Agent	5-20% Ethanol
SS-051D Aerospray® Cytology Hematoxylin I Stain	20-35% Ethylene Glycol
	1-5% Aluminum Sulfate Hydrate
	1-2% Acetic Acid
SS-051D2 Aerospray® Cytology Hematoxylin II Stain	20-35% Ethylene Glycol
	1-5% Aluminum Sulfate Hydrate
	1-5% Acetic Acid
SS-051E Aerospray® Cytology Alcohol Wash	45-55% Ethanol
	45-55% 2-Propanol
SS-051F Aerospray® Cytology Acid Alcohol Wash	50-100% 2-Propanol
	1-4% Acetic Acid
SS-133 Decontamination Solution Concentrate contains:	35-57% Germicidal Detergent
	65-43% Deionized Water
SS-133 Decontamination Solution when	<1% Germicidal Detergent
diluted as directed contains:	>99% Deionized Water

## **Appendix B: Stain Information**

#### Stain Information

#### Stain Description

The stains listed in this manual are for use with the Aerospray Cytology Slide Stainer/ Cytocentrifuge for use by medical professionals to stain specimens as a step of standard laboratory practice in diagnosing disease.

#### Stain Composition

Critical components of stains and cleaning solutions used with this instrument are listed in Appendix A.

#### Storage and Shelf Life

Stains and cleaning solutions are stable up to the expiration date indicated on the label.

Stains and cleaning solutions should be stored 15 – 30 °C unless otherwise stated on the label.

Once opened, stains are stable for 90 days on board the instrument.

#### Hazards and Precautions

The stains and cleaning solutions used with the Aerospray Cytology Slide Stainer/Cytocentrifuge have been classified according to the following standards:

- Globally Harmonized System (GHS) United States Classification
- Regulation (EC) 1272/2008 Classification, Labelling and Packaging of Substances and Mixtures (CLP)

Information for each stain and cleaning solution regarding signal words, hazard classification, hazard pictograms, hazard and precautions statements can be found in the applicable Safety Data Sheet (SDS) for each stain or cleaning solution as well as the product labeling.

SDS for all stains and cleaning solutions can be requested from ELITechGroup Technical Service or can be obtained by accessing the following website:

https://www.elitechgroup.com/documentation

## **Appendix C: Accessories and Supplies**

Only replacement parts supplied by ELITechGroup should be used in this instrument. Use of non-approved parts may affect the performance and safety features of this product. For supplies associated with cytocentrifugation see the user's manual for the Cytopro Cytocentrifuge Rotor (57-2007-01).

Accessories	Reference Number
Drain Tube, 1.8 meter (6 foot) Length	AC-041
Cytopro Cytocentrifuge Rotor	AC-160
10 L Waste Container (without level detect)	AC-170
1D Barcode Scanner	AC-181
10 L Waste Container (with level detect)	AC-182
5 L Reagent Bottle (with level detect)	AC-183
2D Barcode Scanner	AC-185
Slide Carousel with plugs (12-Slide Capacity)	AC-195
Slide Carousel with plugs (30-Slide Capacity)	AC-196
Stain F Empty Bottle with Level Detect	AC-197
Nozzle Cleaning Solution, 355 mL	SS-029
Nozzle Cleaning Solution Concentrate, 250 mL (Dilutes to 500 mL)	SS-029C
EA-50 Stain, 500 mL bottle	SS-051A
Orange G Stain, 500 mL bottle	SS-051B
Bluing Agent, 500 mL bottle	SS-051C
Hematoxylin I, 500 mL bottle	SS-051D
Hematoxylin II, 500 mL bottle	SS-051D2
Alcohol Wash, 500 mL bottle	SS-051E
Acid Alcohol Wash, 500 mL bottle	SS-051F
Decontamination Solution Concentrate, (Dilutes to 250 mL)	SS-133
Supplies	Reference Number
Nozzle Tool	AC-034
Nozzle Hex Wrench	AC-035
Nozzle Orifice Cleaning Wire	AC-059
Reagent Pump Priming Tool	AC-069
Aerospray/Cytopro Safety Shield	AC-110
Nozzle Maintenance Kit	AC-184
O-Ring/Nozzle Thread Grease (3 grams)	SS-103
Preventive Maintenance Chart, pad of 24 sheets (for 7522)	SS-274
Replacement Parts (RP)	Reference Number
Nozzle with mixing insert	RP-499
Paper Test Slides	RP-500
Aerospray Cytology (Model 7522) User Manual	RP-544
Cytopro Cytocentrifuge Rotor (Model AC-160) Applications Manual	RP-517

Contact ELITechGroup for a complete list of replacement parts.

## D.1 Staining Sequence

Table 13: Staining Sequence

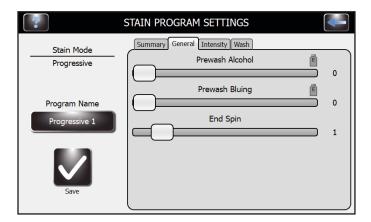
**NOTE:** Table 13 represents the staining sequence with program settings with an approximate stain cycle duration in minutes:seconds.

Bragram Stan		Progressive			Regressive				
Program Step	1:1	5:5	9:9	1:1	5:5	9:9			
Prewash Alcohol	0	0	0	0	0	0			
Prewash Bluing	0	0	0	0	0	0			
Nuclear/Htox	1	5	9	1	5	9			
Acid Alcohol Wash	0	1	3	1	5	7			
Bluing	1	2	3	1	2	3			
Bluing Wash	2	3	5	2	4	7			
Orange G	1	5	9	1	5	9			
Orange G Wash	2	3	5	2	5	8			
Cytoplasmic/EA	1	5	9	1	5	9			
Final Wash	2	3	6	2	5	8			
End Spin	1	1	1	1	1	1			
	Approxi	mate Stain Cyc	le Duration (m	inutes:seconds	5)				
12-Slide Carousel	4:39	8:31	19:30	4:53	9:15	22:38			
30-Slide Carousel	7:05	12:30	28.03	7:19	13:35	32.03			

### D.2 Stain Program Settings

The following is an explanation of the user-adjustable staining parameters and how staining can be affected by adjusting these parameters. Each step has 9 possible settings and 0, which turns the step off.

#### **General Settings**



Carbowax is a highly alcohol and water-soluble component of spray fixatives that must be removed before staining can successfully occur. Because the aqueous hematoxylin reagent used in the ELITechGroup staining process is sufficient to remove the carbowax under most conditions. This is the default setting of the instrument.

#### Prewash Alcohol

This setting adds a series of alcohol applications to remove the carbowax fixative.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	3	3	4	4	4	5	5	7	9
Wait Applications	0	2	0	2	3	0	3	6	9

#### Prewash Bluing

This setting adds a series of bluing applications (which are aqueous based) to remove the carbowax fixative. Since it is aqueous based it can be used on bloody samples to help create a lysing affect.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	3	3	4	4	4	5	5	7	9
Wait Applications	0	2	2	2	3	0	3	6	9

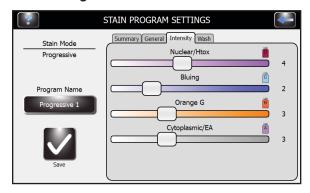
#### **End Spin**

Excessive drying can cause artifacts. Default setting of 1 is recommended to remove excess fluid from the carousel to avoid dripping, but limits the drying.

Stain Setting	1	2	3	4	5	6	7	8	9
Seconds	Х	2	4	10	10	20	30	60	90
RPM	Х	600	600	600	950	950	950	950	950

X = Shake to remove excess fluid from the carousel.

#### Intensity Stain Settings



#### Nuclear (Hematoxylin)

The greater the numeric value selected, the more intense the application. Low settings require less time than higher settings. These settings allow you to vary the staining results according to specimen thickness, type of cells to be stained, or special situations. Refer to Section 13 Appendix E: Sample/Slide Preparation Guidelines for sample treatment guidelines.

The higher the number the darker the stain, the longer the cycle and the more reagent used.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	5	5	6	8	9	10	12	16	20
Wait Applications	6	7	9	12	22	26	35	51	67

#### Bluing

Increase numbers to better blue the hematoxylin in the nucleus. To conserve reagent, select minimum value which produces satisfactory results.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	6	7	8	9	11	13	17	19	23
Wait Applications	2	3	7	11	15	19	27	31	39

#### Orange G

Increase/decrease numbers to strengthen/reduce orange color of keratinized cells. While automatically set by the cytoplasmic intensity, it can be varied independently.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	4	5	6	7	9	11	15	20	25
Wait Applications	6	9	12	15	24	29	39	54	72

#### Cytoplasmic (Orange G and EA)

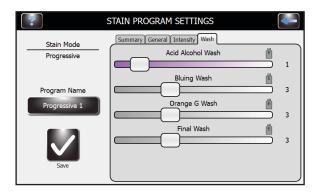
The greater the numeric value selected, the more intense (more stain applied over a longer period) the application. Low settings require less time than higher settings. These settings allow varying the staining results according to specimen thickness, type of cells to be stained, or special situations. Refer to Appendix E for sample treatment guidelines.

**NOTE:** If restaining coverslipped slides, the coverslipping and mounting media must first be removed by soaking in xylene or xylene substitute.

The higher the number the darker the stain, the longer the cycle and the more reagent used.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	4	6	7	8	10	12	16	21	26
Wait Applications	4	12	15	18	24	30	42	57	72

#### Wash Settings



#### Acid Alcohol Wash

Increase numbers to reduce hematoxylin background staining. To conserve reagent, select minimum value which produces satisfactory results.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	1	2	3	3	4	4	6	6	7
Wait Applications	0	0	0	3	4	7	16	15	16

#### Bluing Wash

Increase numbers to better blue the hematoxylin in the nucleus. To conserve reagent, select minimum value which produces satisfactory results.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	2	3	4	5	6	7	8	9	10
Wait Applications	0	0	0	0	0	0	0	0	0

#### Orange G Wash

Removes excess OG reagent in preparation for application of EA reagent. To conserve reagent, select minimum value which produces satisfactory results.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	2	3	4	5	6	7	8	9	10
Wait Applications	0	0	0	0	0	0	0	0	0

#### Final Wash

Removes excess EA reagent at end of staining. To conserve reagent, select minimum value which produces satisfactory results.

Stain Setting	1	2	3	4	5	6	7	8	9
Spray Applications	9	12	12	15	15	21	27	33	45
Wait Applications	0	0	0	0	9	21	36	48	72

Table 14: Summary – Timing Table

Dua waa Otaw	***************************************	Stain Program Settings									
Program Step	*Applications	1	2	3	4	5	6	7	8	9	
Prewash Alcohol	Spray Applications	3	3	4	4	4	5	5	7	9	
	Wait Applications	0	2	0	2	3	0	3	6	9	
Prewash Bluing	Spray Applications	3	3	4	4	4	5	5	7	9	
	Wait Applications	0	2	2	2	3	0	3	6	9	
Nuclear/Htox	Spray Applications	5	5	6	8	9	10	12	16	20	
	Wait Applications	6	7	9	12	22	26	35	51	67	
Acid Alcohol Wash	Spray Applications	1	2	3	3	4	4	6	6	7	
	Wait Applications	0	0	0	3	4	7	16	15	16	
Bluing	Spray Applications	6	7	8	9	11	13	17	19	23	
	Wait Applications	2	3	7	11	15	19	27	31	39	
Bluing Wash	Spray Applications	2	3	4	5	6	7	8	9	10	
	Wait Applications	0	0	0	0	0	0	0	0	0	
Orange G	Spray Applications	4	5	6	7	9	11	15	20	25	
	Wait Applications	6	9	12	15	24	29	39	54	72	
Orange G Wash	Spray Applications	2	3	4	5	6	7	8	9	10	
	Wait Applications	0	0	0	0	0	0	0	0	0	
Cytoplasmic/EA	Spray Applications	4	6	7	8	10	12	16	21	26	
	Wait Applications	4	12	15	18	24	30	42	57	72	
Final Wash	Spray Applications	9	12	12	15	15	21	27	33	45	
	Wait Applications	0	0	0	0	9	21	36	48	72	
End Cain	Seconds	Х	2	4	10	10	20	30	60	90	
End Spin	RPM	X	600	600	600	950	950	950	950	950	

<sup>\*</sup>Spray Application – A single spray application sprays fluid onto the carousel for 1 revolution.

End Spin – X = Quick 100 rpm counterclockwise and clockwise spins to remove excess fluid.

<sup>\*</sup> Wait Application – A single wait application is a slow spin for approximately 1 revolution of the carousel.

## D.3 Examples of Program Settings with Staining Results

Following are examples of buccal specimens prepared using a smear technique and stained using different program setting. The sides were cleared and then cover slipped and viewed at a magnification of 10x.

Table 15: Program Settings with Staining Results 1 and 2

Program Setting	Figure 1	Figure 2
Prewash Alcohol	1	1
Prewash Bluing	0	0
Nuclear/Htox	1	1
Acid Alcohol Wash	3	9
Bluing	1	1
Bluing Wash	2	2
Orange G	1	1
Orange G Wash	2	2
Cytoplasmic/EA	1	1
Final Wash	2	2
End Spin	0	0

Figure 1



Figure 2

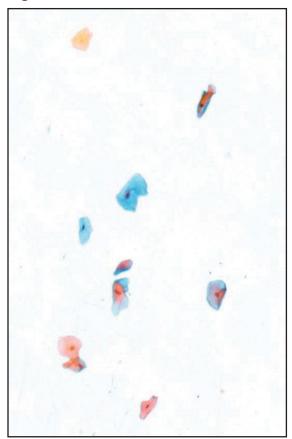


Table 16: Program Settings with Staining Results 3 and 4

<b>Program Setting</b>	Figure 3	Figure 4
Prewash Alcohol	1	1
Prewash Bluing	0	0
Nuclear/Htox	3	7
Acid Alcohol Wash	3	3
Bluing	1	3
Bluing Wash	2	4
Orange G	3	7
Orange G Wash	2	4
Cytoplasmic/EA	3	7
Final Wash	2	6
End Spin	0	0

Figure 3

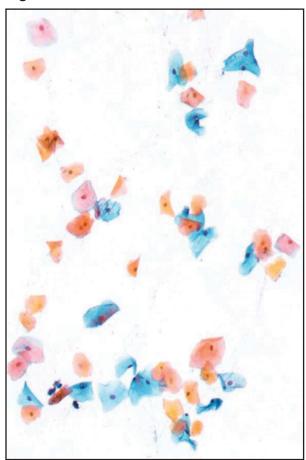


Figure 4

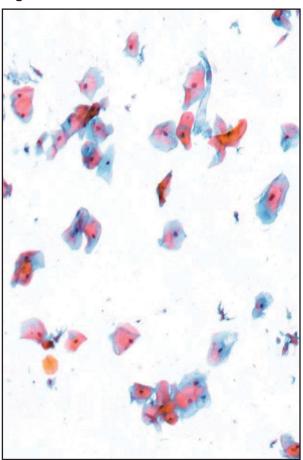


Table 17: Program Settings with Staining Results 5 and 6

Program Setting	Figure 5	Figure 6
Prewash Alcohol	1	1
Prewash Bluing	0	0
Nuclear/Htox	9	9
Acid Alcohol Wash	3	9
Bluing	3	3
Bluing Wash	5	5
Orange G	9	9
Orange G Wash	5	5
Cytoplasmic/EA	9	9
Final Wash	6	6
End Spin	0	0

Figure 5

Figure 6



## **Appendix E: Sample/Slide Preparation Guidelines**

### D.4 General Guidelines

- · Load specimens of similar type in the same carousel.
- Since cytocentrifuged body fluid preparations are deposited in a monolayer, stain intensity setting should be less than that for a smear.
- Higher settings are best for smears since it takes longer for the stain to penetrate to the bottom of a multi-layer of cells.

## D.5 Staining Cytocentrifuged Preparations

NOTE: For best results, always use coated slides (poly-L-lysine SS-118)

Use one of the following methods of fixation:

• Use spray fixative and allow to air dry for a minimum of 15 minutes.



- Immerse in 95% alcohol for up to 30 minutes, but no less than 10 minutes.
- Immerse in a Sacammanno type fixative for up to 30 minutes but no less than 15 minutes.



**NOTE:** If cytocentrifuged sample display area is thick with cells, the probability of the sample being dislodged is very high. A new cytoprep with a cell count decreased would be advisable.

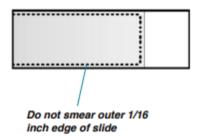
#### Suggested Staining Settings

- 1. Use the No Pre-Wash setting.
- 2. Use lower Nuclear/Htox intensity setting.
- 3. Use lower Cytoplasmic/EU intensity setting.

## Appendix E: Sample/Slide Preparation Guidelines

### D.6 Staining Smears

1. Avoid spreading smear on the edges of the slide.



- 2. Recommend using coated slides (SS-118).
- 3. Fix smears using one of the following methods:
  - ♦ Spray fixative.
  - ♦ Immerse in 95% alcohol for 15 to 30 minutes.
  - ♦ Immerse in a Sacammano type fixative for 15 to 30 minutes.
- 4. Suggested settings:

♦ Nuclear/Htox: 3, 4, 5, 6

♦ Cytoplasmic/EU: 3, 4, 5, 6

♦ No Pre-Wash

### D.7 Alternative Carbowax Removal Method

Commercially available spray fixatives have varying amounts of polyethylene glycol (carbowax). Carbowax protects cells from air drying before fixation but can inhibit staining if not completely removed. Because the aqueous hematoxylin reagent used in the ELITechGroup staining process is sufficient to remove the highly water soluble carbowax under most conditions, the no Pre-Wash (default) settings are usually acceptable. If the specimen needs to be protected from cell loss, the following method may be preferable to the lower or higher Pre-Wash settings of the instrument:

#### To Remove Carbowax

1. Soak slides in 95% alcohol or 50% glycerol / 50% alcohol, for 10 to 30 minutes before staining.



## **Appendix F: Cleaning Solutions**

ELITechGroup Inc. offers several cleaning solutions for the Aerospray Stainer/ Cytocentrifuge family. The following products are available to keep your Aerospray running safely and optimally.

#### SS-029 and SS-029C/SS-029C-EU Aerospray® Nozzle Cleaning Solution

Aerospray Nozzle Cleaning Solution (SS-029) and Aerospray Nozzle Cleaning Solution Concentrate (SS-029C/SS-029C-EU) when diluted as recommended should be used for cleaning the instrument. Specifically for:

- General cleaning
- Nozzle cleaning
- Instrument interior and exterior cleaning
- Carousel cleaning

The Aerospray Nozzle Cleaning Solution may be purged through the instrument pumps without causing damage to the instrument.

Dilution instructions for the Aerospray Nozzle Cleaning Solution Concentrate (SS-029C/SS-029C-EU) can be found by referring the instructions in **DOC-01301**.

#### SS-133 Decontamination Solution Concentrate

Decontamination Solution Concentrate (SS-133) when diluted as recommended should be used for decontamination of the inner and outer surfaces before the instrument is returned to ELITechGroup Inc. for Service or when instrument will be prepared for long-term storage.

#### Introduction

George Papanicolaou developed what now is known as the "pap stain" in the early 1940's (1-3) during his studies to detect the action of ovarian hormones on vaginal specimens. Its origins lie in the earlier staining methods of Shorr and Mallory as reviewed by Marshall (4).

Over the years, the papanicolaou stain has attained a pre-eminent position in the analysis of gynecological smears and body fluids. The procedure employs hematoxylin as the nuclear stain with Orange G, Eosin Y and light or fast green SF as counterstains for cytoplasmic elements. Bismark brown was originally included but was subsequently shown to be superfluous <sup>(4)</sup>.

The papanicolaou stain is designed to meet three staining objectives; good nuclear detail, differential counterstaining and cytoplasmic transparency.

First, the nucleus is stained with hematoxylin, fulfilling the first objective of good nuclear detail. The hematoxylin has an affinity for chromatin and RNA-rich cytoplasm. If the cell is quickly and properly fixed the nucleus will present a fine granular appearance. Depending on the hematoxylin formulation used, either a regressive or progressive staining method is employed.

The counterstains provide the differential staining of cells to display their maturity and metabolic state. This differential staining is partially a function of stain penetration, which depends on the size of the stain molecule and the density of cellular structure (4,5,6).

Orange G, being the smallest of the three (MW = 452), is able to penetrate dense keratinized cells to provide a yellow-to-orange color. Eosin Y (MW = 692) competes with the Orange G in the superficial and other cells which it is able to penetrate within the staining period. This results in the superficial cell generally being stained pink to red.

Light green SF (MW = 793) primarily penetrates the least dense intermediate cells within the staining period to stain them blue to green. In addition to the dye size in determining cell differentiation, staining is also dramatically affected by pH <sup>(5,6)</sup>, presumably by altering electrostatic interactions. In addition, a mordanting effect of phosphotungstic acid for light green has been described <sup>(6,7)</sup>, and other mechanisms may also be involved <sup>(4)</sup>.

The cytoplasmic transparency is a function of the high ethanol content of the stain. Cytoplasmic transparency is important in order to view multi-layered cell aggregates. A substantial number of variations of the papanicolaou stain have been formulated. The most useful probably being the elimination of Bismark brown which does not appear to stain any component of Pap smears <sup>(8,9)</sup>, and can form precipitates with PTA <sup>(10,11)</sup>.

A combined OG-EA stain was developed <sup>(12)</sup>, replacement of hematoxylin with thionin was proposed <sup>(13)</sup> and several quick stains have been formulated <sup>(14-17)</sup>. Current commercial practice still focuses on minor variations of the traditional procedures. Hematoxylin, OG and EA stains of improved stability and reproducibility are currently offered. Several formulations of the EA stain, i.e. EA-50, EA-65, are offered. These differ primarily in the concentration of eosin and fast or light green.

Due to the wide range of commercial and private formulations available, the stain is far from standardized. Formula selection depends primarily on personal or regional preferences. The EA-50 solutions ELITechGroup provides are filtered through a 0.2 µm filter to provide particle-free staining solutions.

#### The ELITechGroup Staining System

In adapting the traditional papanicolaou stain to the Aerospray stainer, several benefits have been incorporated which provide more flexibility in the papanicolaou staining procedure:

#### 1. No Cellular Cross-Contamination

Since only fresh unused stain is applied to the slides, cellular cross-contamination from dislodged cells in the stain baths is impossible. This benefit eliminates the need to filter stains to rid them of sloughed off cells. Because each slide is individually stained on a rotating carousel, it is virtually impossible for cells that may dislodge from slides to deposit on another slide. Varying specimens can be stained in the same carousel without cellular cross-contamination but are usually separated because of differing staining

requirements. Since a small volume of stain is applied, stain usage is comparable to dip staining.

#### 2. No Dilution

Stain concentration does not vary with time due to evaporation or dilution with carryover alcohol and loss during daily filtering procedures as in the traditional dip method. The wash alcohol is always fresh and uncontaminated of stain or cell debris. Alcohol usage is reduced up to 50% compared to the dip method of staining.

#### 3. Centrifugal Clearing

The Aerospray Cytology employs a centrifugal clearing to remove excess reagents. See **Stain Chemistry - Clearing and Mounting** below for more information.

#### 4. Throughput

Through optimization of the staining times required, and employing spray washes, the cycle time for the Aerospray Cytology is approximately 4.5 to 32 minutes. This is ideal for quick turnaround times. Using the default settings the instrument is shipped with, the instrument has a capacity of about 80 slides per hour using the 12-slide carousel, and 120 slides per hour when using the 30-slide carousel.

#### Stain Chemistry

#### 1. Hematoxylin Stain

Hematoxylin is a natural product extracted from the logwood tree of South America. It is not a dye but is oxidized to hematein. which is the staining principle when combined with a suitable mordant such as aluminum to form AL- hematein.

The oxidation can be accomplished with a wide variety of oxidants <sup>(4)</sup>. If hematoxylin is overoxidized, oxyhematein is produced. This is considered to be a cause of stain degradation and contributes to poor nuclear staining. Another mechanism of stain degradation is precipitation. Most hematoxylin stains are aqueous solutions of aluminum hematein. Since hematein is only sparingly soluble in water, this leads to precipitation of the active ingredient and hence, a weakened stain concentration.

The Gill formula <sup>(19)</sup>, is a popular modern commercial stain. It recognizes Baker's observation that hematein is much more soluble in ethylene glycol than water <sup>(20)</sup>. It employs half oxidized hematoxylin according to Baker and Jordan <sup>(21)</sup>, and the capabilities of a high-contrast progressive stain as described by Cole <sup>(22)</sup>. The attractive properties <sup>(23)</sup> of a mordant quotient of 8-16 is also provided.

ELITechGroup offers two concentrations of a Gill type formulation, providing excellent long-term stability. It is formulated by actual hematoxylin content as measured by the method of Marshall and Horobin (24), since commercial hematoxylins can vary considerably in purity. The Gill hematoxylin is used in a progressive staining method yielding fine nuclear detail.

#### 2. Bluing Agent

In order to "blue" the hematoxylin, the pH of the cellular environment must be raised to above 5.0. This can be accomplished with a variety of bluing agents—even distilled water of the correct pH (20). The Aerospray employs a dilute solution of sodium bicarbonate which can also be used for hydrating the cells prior to hematoxylin staining. Since the molarity of the rinse is low, the acidity of the hematoxylin solution overpowers the weak buffering capacity of the rinsing agent until the hematoxylin solution is essentially removed from the slides. In addition, the low molarity avoids the need to remove any excess bluing agent by a water wash. This unique modification of the ELITechGroup papanicolaou stain avoids the need for a separate water washing system and shortens the staining time.

A second unique modification to the bluing reagent is the 10% alcohol content. This low percentage of alcohol helps to reduce the forces exerted on the slide when it is subsequently exposed to 100% alcohol for the dehydration series prior to the OG stain.

#### 3. Orange G Stain

Orange G is a small, negatively-charged dye important to the papanicolaou stain for visualizing keratinized tissue. It also enhances the EA stain by acidification of the smear <sup>(6)</sup>.

ELITechGroup employs an OG stain as in the traditional papanicolaou stain. The OG stain is filtered using an 0.2 μM filter to eliminate any precipitates.

#### 4. Alcohol Wash

The traditional papanicolaou stain procedure employs a graded alcohol series of 50, 75, 95%, for washing and dehydration. This has been shown to be unnecessary <sup>(12)</sup>. Dunton recommends anhydrous isopropanol, as it has a lower capacity to destain the smear during washing. Normally ethanol is employed.

Reagent E provides a better destaining action for cells and also for the nozzles and carousel during the Clean Cycle and is more miscible than ethanol in most mountant media.

#### 5. EA-50 Stain

The ELITechGroup EA stain is an alcoholic solution of Eosin Y and fast green or light green SF. Phosphotungstic acid and acetic acid are employed to enhance the binding of the green component. The solution is designed to provide staining comparable to other commercial EA stains. Bismarck brown is omitted as previously discussed.

The Aerospray stainer provides 9 settings which alter the amount of stain applied and the time of staining. Since the differentiation between cells is at least partially based on staining rate, it is affected by the setting employed. The short setting provides little staining by fast green (or light green). As the stain setting is increased, the green predominates, and the smear becomes darker and bluer. The modification of the staining balance, however, is somewhat limited using the settings alone. Greater alterations require modification of the staining solutions. When used without modification, the stain yields a turquoise intermediate cell and pink-to-red superficial cells. This can be modified as described by Boon and Drijver<sup>(5,6)</sup>. Raising the pH enhances the blue color and lowering the pH shifts the color to green.

#### 6. Acid Alcohol Wash

The Acid Alcohol Wash is designed to clean and maintain the Hematoxylin Reagent D line.

#### 7. Clearing and Mounting

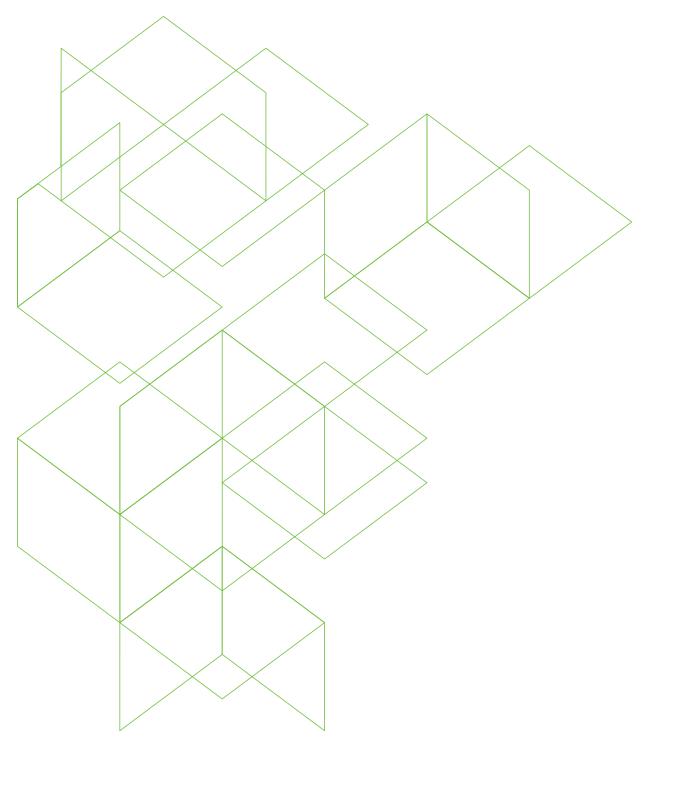
The traditional method of clearing is to replace residual alcohol with an aromatic hydrocarbon (commonly toluene or xylene). The Aerospray Cytology employs a centrifugal clearing to remove excess reagents. This allows alternative clearing solvents (such as Formula 83), free of the toxicities associated with hydrocarbons, to be used. Other organic solvents may also be used, Drijver and Boon prefer t-butanol (18).

The Aerospray Cytology avoids the need for xylene use by washing the slides with fresh anhydrous alcohol coupled with centrifugal clearing. However, xylene or toluene may offer an advantage in certain high humidity environments.

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