Automating Special Stains Using a Commercial Autostainer

Rachel Peters
Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada

Abstract

A commercial automatic stainer was successfully used to automate 2 versions of the hematoxylin and eosin stain as well as 10 special stains frequently performed in an histology laboratory. The special stains included Van Gieson, diastase/periodic acid Schiff (PAS), PAS, alcian blue, alcian blue/PAS, Gomori 1-step trichrome, alcian yellow/toluidine blue, Schmorl, Perl prussian blue, and luxol fast blue.

The capabilities of the existing hardware and software of the Leica Autostainer XL were explored and some of the hardware was modified: the staining rack adaptor was used to accommodate the staining racks used in this laboratory, and an insert was designed to fit in the autostainer's forced air oven so it could be used for staining purposes rather than as a forced-air slide dryer. Limitations of the software were overcome by timely exit and re-entry at certain steps in some of the staining programs.

The modifications expanded the functions of the autostainer. As a result, multiprogram compatibility was obtained for a larger variety of special stains than were ever previously programmed on this automated slide stainer. (*The J Histotechnol* 21:135, 1998)

Key words: automated staining hydrating and de-hydrating center, oven insert, staining program compatibility, staining rack adaptor

Introduction

In spite of the success achieved in automating some special and immunohistochemical stains, automation of a wide variety of special stains has not been pursued, neither by the manufacturers of the various staining machines nor histotechnologists. A fully automated special stains bench would be most useful, particularly during this period of hospital restructuring in which a reduced staff is expected to perform with the same efficiency as a larger staff.

In an attempt to automate special stains frequently requested in our laboratory, I researched several of the commercially available automated stainers. Although many of these stainers were capable of being programmed with several staining methods, they were able to perform only 1 stain at a time. Because the Leica Autostainer could be programmed for 15 stains and able to perform 11 compatible stains simultaneously, I selected this autostainer as the most technologically suitable.

This paper reports my experience with automating a large selection of special stains using a multi-function automated slide stainer. The goal was to determine whether automating special stains was feasible, could be standardized, and increase productivity.

Materials and Methods

Leica Autostainer XL (Figure 1a)

An automated slide stainer capable of being programmed with 15 different stains but capable of simultaneously performing only 11 compatible staining protocols was used (4).

Tissues

Approximately 100 paraffin sections of surgical and autopsy tissue previously fixed in 10% neutral buffered formalin, cut at 3 μm to 5 μm, were used for the initial tests. These included 5 μm sections of lymphoid tissue fixed in B5 fixative to test the hematoxylin and eosin stain with iodine pre-treatment for the removal of mercury pigment and, in addition, known positive control sections for iron, melanin, glycogen, mucin, and the micro-organism *Campylobacter pylori*.

Reagents

The following solutions were prepared: 1% periodic-acid, 1% acid alcohol, 1% acetic acid, 0.1% acetic acid, 2% aqueous malt diastase (BDH Inc, Poole, England, # 39013), Lugol iodine, and 1% sodium hypochlorite. A commercial preparation of Bouin fluid (BDH, #RO3290) was also used.

Staining Solutions

Commercial preparations of Harris hematoxylin (EM Diagnostics, Gibbstown, NJ, #HOO 638-83), Schiff reagent

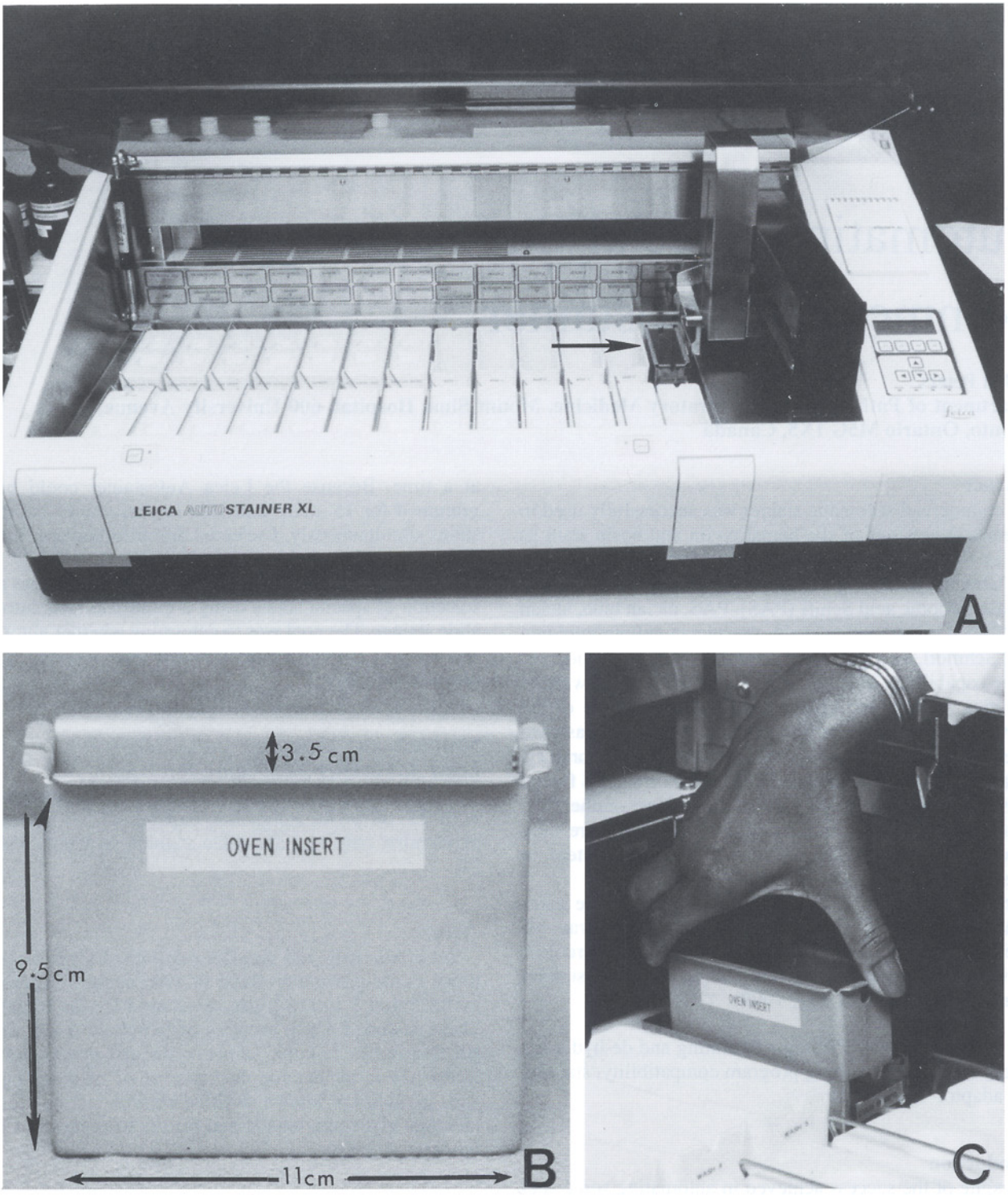


Figure 1. **(A)** Leica Autostainer XL, shown with oven insert →. **(B)** Oven insert designed to fit inside the autostainer oven. **(C)** Oven insert being placed into the autostainer oven.

(Sigma, St Louis, MO, # S-5133), and 1% eosin (BDH, # RO3378) were used. The following staining solutions were prepared: 1% alcoholic alcian yellow in 3% acetic acid, 1% aqueous toluidine blue, 0.5% luxol fast blue in methanol with acetic acid, celestine blue, 1% aqueous alcian blue in 3% acetic acid, Gomori 1-step trichrome stain, Van Gieson stain, 1% aqueous neutral red, Schmorl solution for melanin, and Perl prussian blue reagent for iron (1–3).

Programming

Before programming any stains it was necessary to establish which of the routine special stains would be automated. For multiple staining protocols to work simultaneously, they must be compatible. To achieve multiple program compatibility, 2 critical issues had to be considered. The software on the autostainer would not allow a staining rack to re-enter a staining tub that it had entered

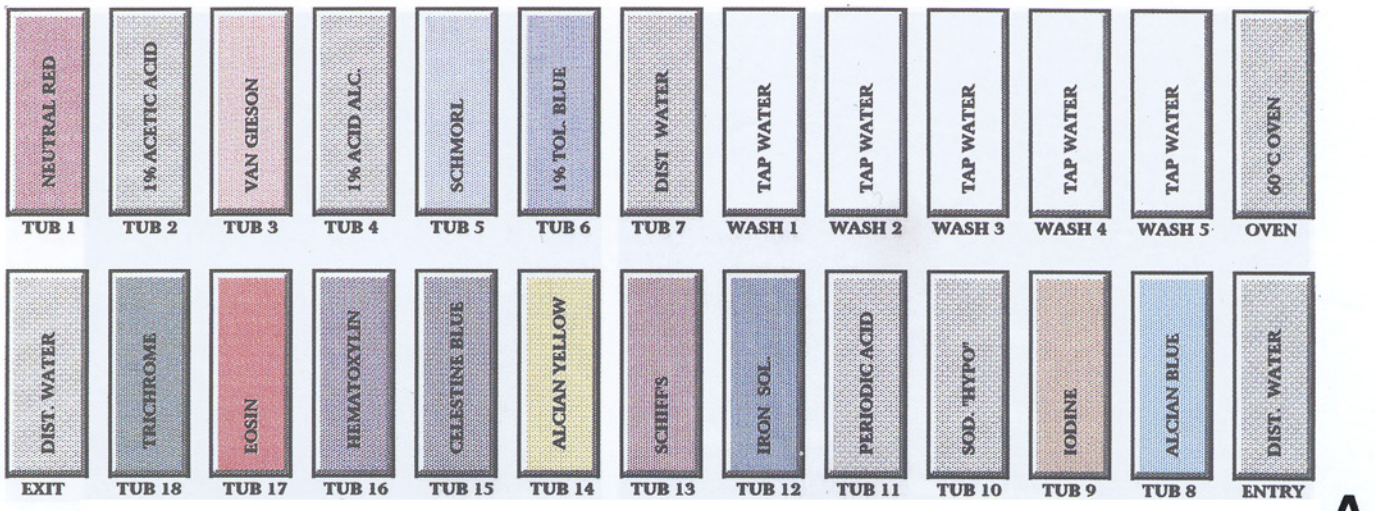
previously in the same program nor allow 2 racks of slides to approach the same staining tub from different direction. To get around these limitations with the software, I used a spreadsheet (Appendices 1 and 2) to sequentially determine the steps of the various staining protocols and to assign stains and reagents to the containers on the Autostainer (Figures 2 a&b).

The AST Premmia MX 486K RAM, IBM-compatible computer was used to input 12 staining protocols onto program optimization software designed for this purpose and provided by Leica on a 1.44M format 3 1/2 inch disk. Once compatibility among the stains was achieved (Table 1) the protocols were downloaded onto the autostainer's software using an IBM-compatible laptop computer connected to the autostainer via a RS232 serial data port (9-pin AT style port COM1:).

The staining times used in the manual methods were used for similar special stains, and the concentrations of solutions and stains were kept exactly as when prepared for manual staining. Staining times used by the automated linear stainer for routine hematoxylin and eosin (H&E) staining were applied to the H&E protocol on the Autostainer (Appendices 3 and 4).

Other Adaptations

Other adaptations included manually hydrating to distilled water (DW) and dehydrating to xylol off the auto-



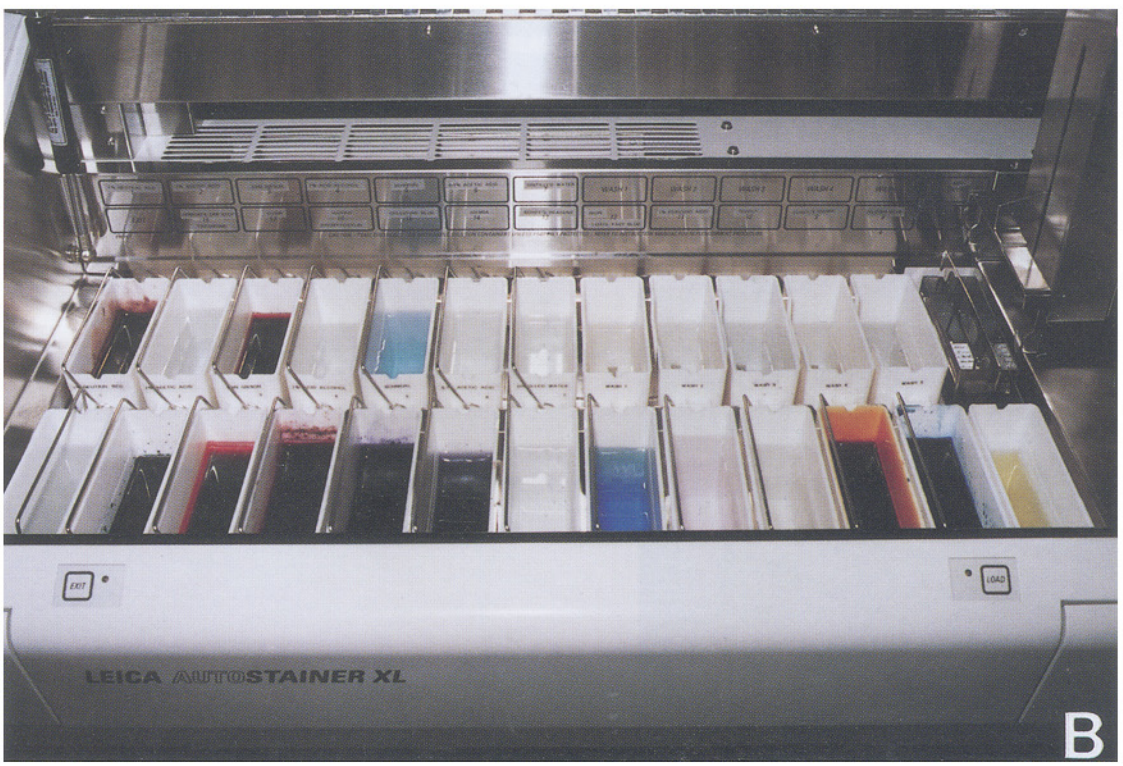


Figure 2. **(A)** Schematic layout of staining tubs. When staining luxol fast blue, exchange tub 12 for one containing the luxol fast blue stain. **(B)** Layout of the staining tubs on the Autostainer XL.

stainer to release more tubs for stains rather than for alcohols and xylols; sharing similar staining stations for 2 compatible staining methods; using the XL's forced-air oven for staining purposes; and using the Leica rack adaptor to accommodate the staining racks on the autostainer.

Hydrating and Dehydrating Center

This area was used to take airdried paraffin sections to running tap water, then into DW in the entry tub on the autostainer, and to dehydrate stained sections taken from DW in the exit tub on the Autostainer. Two xylols, 1 alcohol/xylol, and 3 alcohols were used for hydrating purposes. Dehydration was done by returning, in reverse order, through the same 3 alcohols and the alcohol/xylol; but there were 2 different changes of xylols prior to mounting in Permount (Fisher Scientific, Fair Lawn, NJ).

Oven Insert (Figure 1b)

A metallic container (Marivac, Halifax, Nova Scotia, Canada) was designed so that it could be inserted into the stainer's existing oven. Its purpose was to accommodate staining solutions needing heat (eg, Bouin solution prior to applying Gomori 1-step trichrome stain and the diastase solution prior to the PAS technique) (3).

Leica Staining Rack Adaptor (Figure 3a–B)

The 20-slide staining racks (Figure 3a–A) used in our laboratory are smaller and fit both the staining tubs used for

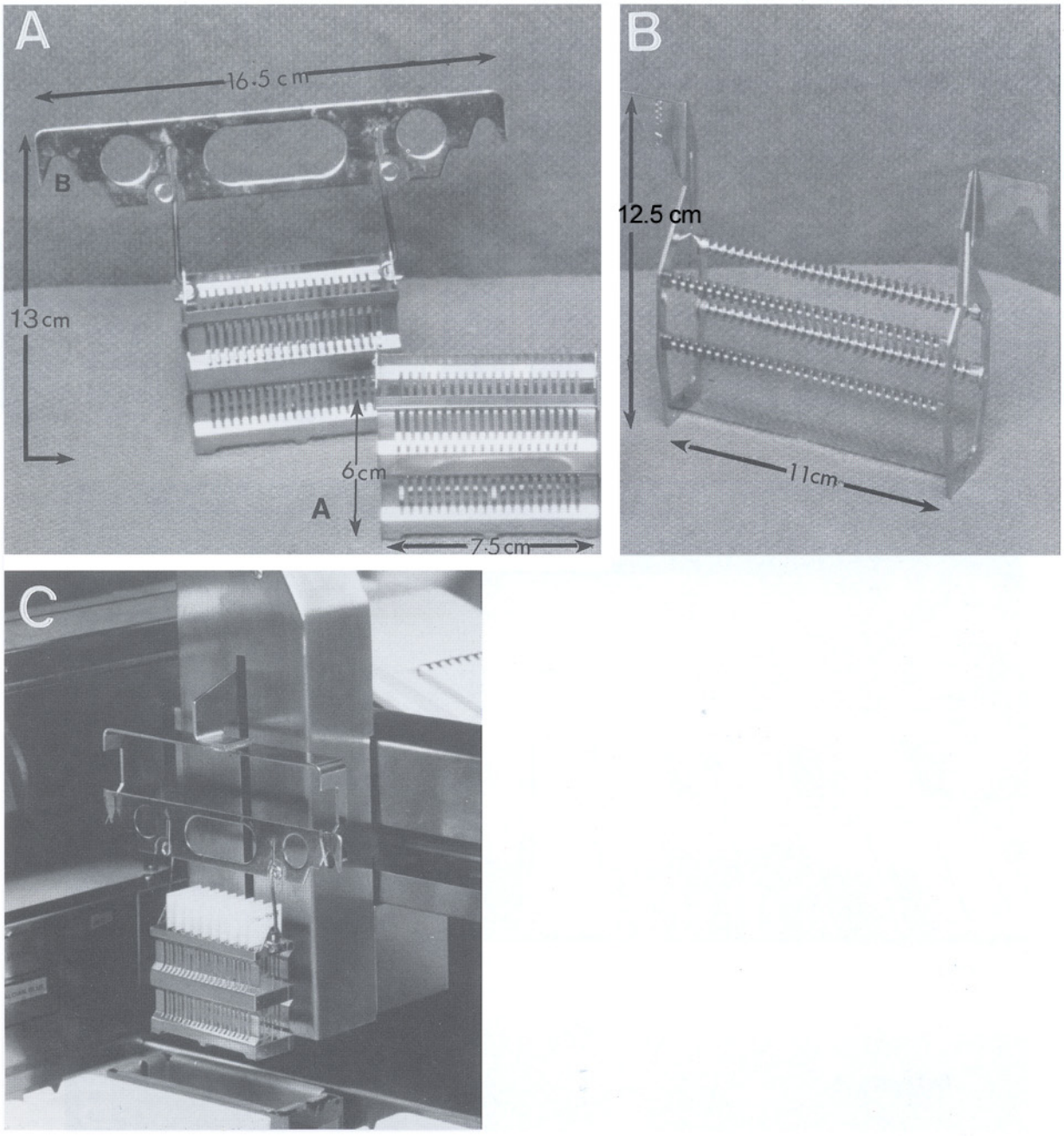


Figure 3. **(A)** A: Our staining rack. B: Leica staining rack adaptor attached to our staining rack. **(B)** Leica staining rack. **(C)** Our staining rack with attached Leica rack adaptor entering oven containing insert.

Table 1. Program Compatibility Check Matrix

Y for Yes, N for No, - for No Program

PROGS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
2	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
3	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
4	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
5	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
6	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
7	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
9	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
10	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
11	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
12	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

manual staining and the tubs used on the automated linear stainer for staining of the routine H&E. Because the larger Leica 30-slide staining racks (Figure 3b) did not fit into the staining tubs routinely used in our laboratory, a compatible system for staining was devised by using the Leica staining rack adaptor to accommodate our 20-slide staining racks on the Autostainer.

Results (Figures 4 A–J)

In order to maintain a standardized process, the results obtained when the same special stains were performed manually were used as references. All the stains when initially programmed, used the same times of the stains when performed manually. For each different staining procedure, I used a slide mounted with 5 different types of tissue, among which were known positive controls for iron, melanin, glycogen and mucin. These tissues comprised skin, colon, liver, kidney, and lymph node. In addition to this, a separate positive control for *C. pylori* was used for the alcian yellow/toluidine blue method, and a section of a lymph node fixed in B5 fixative was used for the H&E method that included the removal of B5 pigment (1).

Initial results from the H&E protocol showed pale nuclei staining after exposure to hematoxylin for 8 min. Adjustments were made solely to the length of time in the hematoxylin stain; 10 min in hematoxylin provided similar results as the H&E from the automated linear stainer. Staining of alcian yellow/toluidine blue, luxol fast blue, alcian blue, PAS, alcian blue/PAS, PAS with diastase pre-treatment, Gomori one-step trichrome, hematoxylin and Van Gieson, iron, and Schmorl provided acceptable results almost immediately (1–3). It took no more than 3 repeats for the more difficult stains such as the Gomori 1-step trichrome, the alcian blue, and the Schmorl to achieve the same results as the manual methods.

In the case of the Gomori 1-step trichrome stain, the rinse with 1% acetic acid was done off the stainer because this afforded more control over differentiation of the dye. With the alcian blue, Schmorl, and Perl prussian blue, the most difficult step was manipulating the length of time in the neutral red stain to achieve the desired results. Sections were stained in neutral red for 2, 3, 4 and 5 min, respec-

Table 2. Comparison Costs for Stains With Non-Reusable Reagents

A. Iron

Method	*Manual*	*Manual*	*Automated*
Number of slides	1 to 5	1 to 20	1 to 20
Cost of reagents	$3.00	$12.80	$25.60
Tech time	18 mins	24 mins	15 mins
Tech cost @ $24/hr	$7.20	$9.60	$6.00
Total cost per run	$10.20	$22.40	$31.60

B. Schmorl

Method	*Manual*	*Manual*	*Automated*
Number of slides	1 to 5	1 to 20	1 to 20
Cost of reagents	$3.06	$12.26	$24.52
Tech time	18 min	24 min	15 min
Tech cost @ $24/hr	$7.20	$9.60	$6.00
Total cost per run	$10.26	$21.86	$30.52

Table 3. Comparison Costs for Stains With Reusable Reagents

A. Periodic acid Schiff:
Reagents discarded when staining by manual method

Method	*Manual*	*Manual*	*Automated*
Number of slides	1 to 5	1 to 20	1 to 20
Cost of reagents	$8.10	$9.20	$36.80
Tech time	12 min	24 min	15 min
Tech cost @ $24/hr	$4.80	$9.60	$6.00
Total cost per run	$12.90	$18.80	$42.80
Cost after 10 runs	$129.00	$188.00	$96.80

B. Gomori 1-step trichrome
All reagents reused

Method	*Manual*	*Manual*	*Automated*
Number of slides	1 to 5	1 to 20	1 to 20
Cost of reagents	$26.55	$90.45	$124.80
Tech time	13 min	22 min	15 min
Tech cost @ $24/hr	$5.20	$8.80	$6.00
Total cost per run	$31.75	$99.25	$130.80
Cost after 10 runs	$78.55	$178.45	$184.80

C. Alcian blue
All reagents reused

Method	*Manual*	*Manual*	*Automated*
Number of slides	1 to 5	1 to 20	1 to 20
Cost of reagents	$4.10	$16.40	$24.00
Tech time	11 min	18 min	15 min
Tech cost @ $24/hr	$4.40	$7.20	$6.00
Total cost	$8.50	$23.00	$30.00
Cost after 10 runs	$48.10	$88.40	$84.00

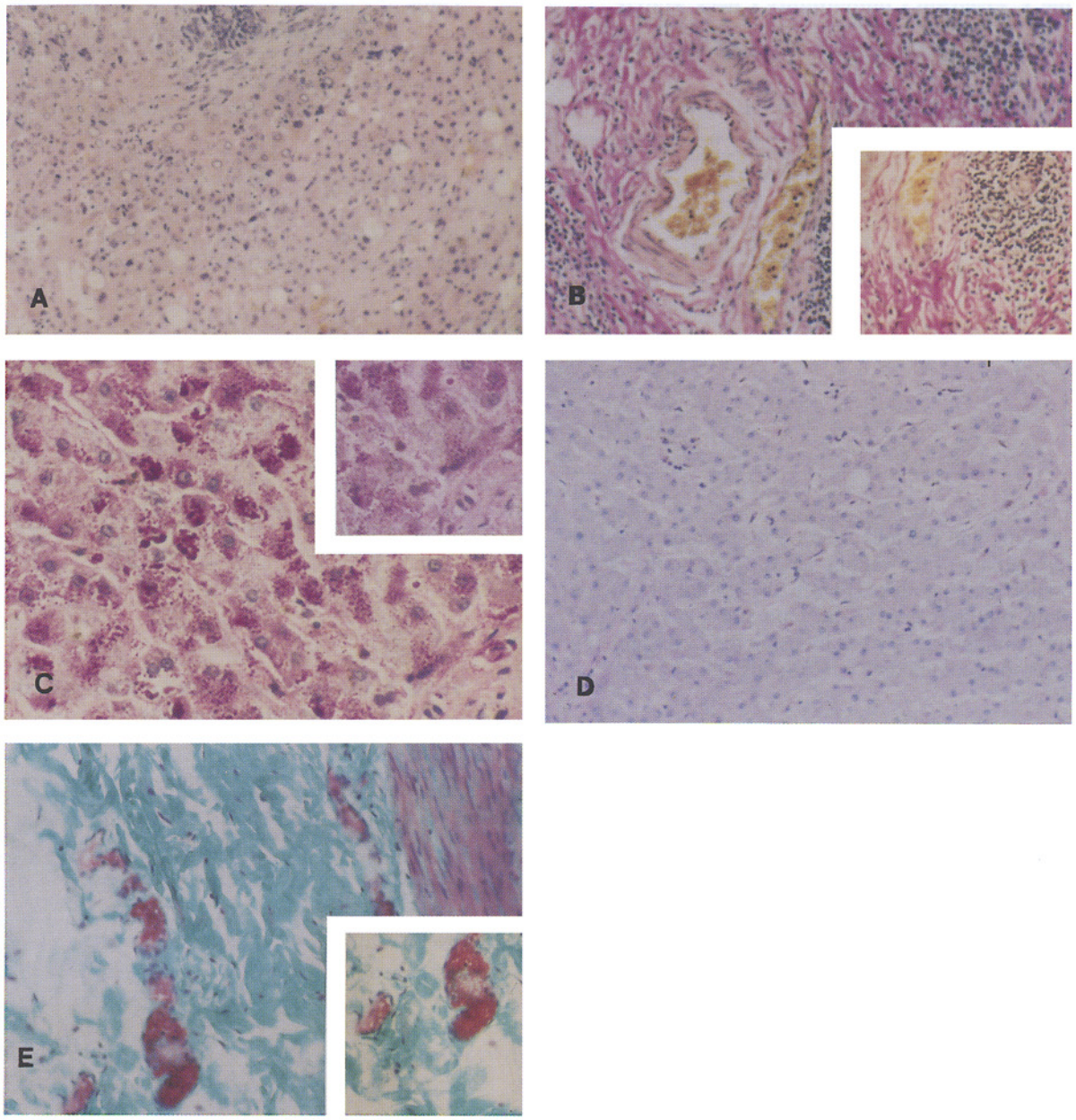


Figure 4. **(A)** H&E, colon. **(B)** Hematoxylin and Van Gieson, colon (inset hematoxylin and Van Gieson manual staining). **(C)** PAS, liver (inset PAS manual staining). **(D)** Diastase/PAS, liver. **(E)** Gomori 1-step trichrome, colon (inset Gomori 1-step trichrome manual staining).

tively, and all were treated with a tap water rinse of 1 min after neutral red. The 5 min exposure in neutral red combined with the 1 min rinse in tap water provided the most acceptable results. The PAS, hematoxylin, and Van Gieson were acceptable after the first try. However to retain the yellow color (picric acid) in the Van Gieson stain, the sections were blotted after they were removed from the stainer, allowed to airdry, and rinsed in xylol prior to mounting in Permount (Fisher Scientific).

Comparison costs demonstrated that the Autostainer may not be cost effective for running stains for which the reagents can only be used for a single batch, eg, Perl prussian blue for iron and Schmorl for melanin (Tables 2 A&B). However, although the reagents may cost more for automated staining because of the volume (400 ml) required to fill the staining tubs, a saving of 9 min per run for technologist time when compared with the same number (20).

For those stains where the reagents are re-usable with frequent filtering and proper storage (at 4–6°C), comparison costs show the cost effectiveness of automation. Reagents for stains such as the periodic-acid Schiff, Gomori one-step trichrome and alcian blue have retained their reactivity after 10 to 12 runs and up to 2 weeks with fewer runs (Tables 3 A, B, C).

Discussion

Budgetary and fiscal constraints have led to many reductions in laboratory staff. In order to perform the work of the Histology Laboratory with a reduced staff and improve the delivery efficiency of laboratory services, automation

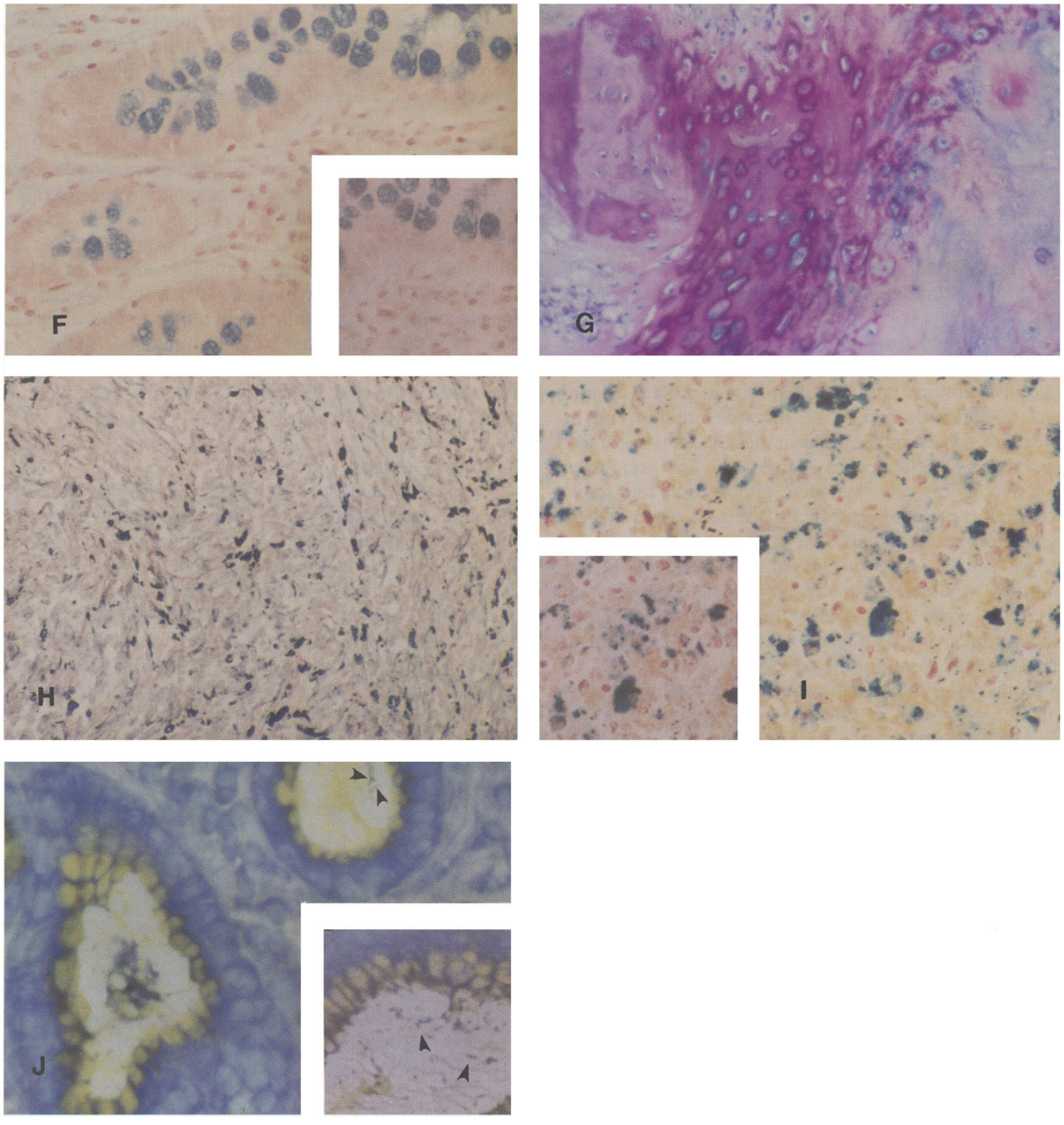


Figure 4. **(F)** Alcian blue, colon (inset alcian blue manual staining). **(G)** Alcian blue/PAS, adenocarcinoma tumor of bone. **(H)** Schmorl for melanin, skin with melanoma. **(I)** Perl prussian blue for iron, liver (inset Perl prussian blue manual staining). **(J)** Alcian yellow/tolouidine blue for *Helicobacter pylori*, gastric biopsy (inset alcian yellow/toluidine blue manual staining). Original magnifications ×25.

was introduced where feasible. One of these areas was the special stains bench. The workload in this area has increased by approximately 22% over the previous year. Projections are that further increases will occur over the next 5 years. The literature has shown that consistently reliable results have been obtained by automating the Papanicolaou stain for cytological preparations, variations of the H&E, and a small number of special stains like the PAS, alcian blue/PAS and toluidine blue using the Leica Autostainer XL (3,4).

Automation at the special stains bench for a larger selection of special stains was therefore seriously considered. I believed that this would provide standardized conditions, decreasing the variations in results obtained when stains are manually performed by different technologists, free the technologist on this rotation to perform other tasks, and make special stains more manageable.

The Leica Autostainer XL was chosen because it was equipped with the technology to satisfy the above situations. The Autostainer's hardware includes 18 reagent tubs, each holding approximately 400 ml of solution. Leica's protocols suggest 8 to 9 of these tubs be used for deparaffinization and hydration, which left 9 to 10 for staining reagents. It also had 5 wash stations, 1 entry station, 1 exit station, 1 forced-

air oven for drying slides with temperature adjustments from OFF to 65°C, a robot arm that delivers a 30-slide rack to all of the 26 stations, a control panel with keyboard and function keys for operating computer programs, and a charcoal filter system.

Shortcomings of the hardware include the inability to use the oven for staining solutions requiring heat; the requirement that the tub handles be laid flat to prevent obstruction of the movement of the robotic arm carrying the slides; the necessity to remove the staining racks promptly and press the "Exit" button (failure to do so would result in overstaining as all further staining processes would be halted); and the large volume of solution (400 ml) required for the staining tubs.

Manipulation and fine-tuning of staining times was the main factor in the testing phase, and quality management of the staining solutions became an on-going process. Large amounts of solutions had to be discarded at the end of certain stains, for example, the iron and Schmorl methods. Fresh solutions of acid alcohol and periodic acid were used daily; hematoxylin, celestine blue, and neutral red stains were filtered every other day; Schiff reagent was refrigerated when not in use; and all other stains were filtered once per week. The large volume of solution required in the staining tubs would render the use of the stainer too costly in laboratories where the special stains workload is light. In such laboratories serious consideration should be given to batching of stains to minimize the costs.

Shortcomings encountered with the autostainer's software made the system a little less user friendly than expected. Among these was the inability to restart a stain at a desired step within a particular staining program. For example, if eosin staining was too pale in the H&E staining protocol, it was not possible to simply return to the eosin step and re-stain for a longer period. To get around this, I had to create a new program starting at eosin. It was also not possible to view the status of programs being run. For example, I could not determine at which step in a particular program the process was, nor could the length of time remaining in a particular stain or stains be determined. Accordingly, the time remaining in the entire staining run could not be ascertained.

The goal of 12 fully compatible stains was eventually achieved through adjustments to the programs, such as exiting and re-entering programs at pre-determined steps in the staining programs. Modification to the hardware was also made, such as the insertion of the metallic container in the oven, the use of the Leica staining rack adaptor, and dehydrating and hydrating off the stainer.

Conclusions

When consideration is given to the current trend towards downsizing of laboratory staff, the manufacturer of this versatile autostainer and all other manufacturers should be aware of histotechnologists' needs. It would be of benefit to all to see in the near future a smaller, more cost effective stainer capable of "performing like a technologist at the special stains bench" while using only one-half of that technologist's time. Until then, histotechnologists should be encouraged to experiment with automation of the diverse special stains pertinent to their laboratories and adapt their autostainers accordingly.

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Appendix 1. Spreadsheet to Determine Staining Sequence

		H&E FOR B5	FIXED BLOCKS		ROUTINE H&E			DIASTASE	PRE-PAS			PAS		PERL	IRON		ALCIAN BLUE		
STATION	REAGENT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT
ENTRY	DISTILLED WATER																		
OVEN @ 60 C	BOUIN'S SOL. / DIASTASE							1 (DIASTASE)	20:00	YES									
1	1% NEUTRAL RED													3	05:00	YES	3	05:00	YES
2	1% ACETIC ACID																		
3	VAN GIESON																		
4	1% ACID ALCOHOL	7	00:10	YES	3	00:10	YES				8	00:10	YES						
5	SCHMORL'S REAGENT																		
6	1% AQ. TOLUIDINE BLUE																		
7	DISTILLED WATER										3	01:00	YES						
WASH 1	TAP WATER	2	02:00	NO				2	03:00	NO	2	02:00	NO	2	05:00	NO			
WASH 2	TAP WATER	4	03:00	NO							5	10:00	NO	4	01:00	NO			
WASH 3	TAP WATER	6	02:00	NO	2	02:00	NO				7	02:00	NO				2	02:00	NO
WASH 4	TAP WATER	8	03:00	NO	4	05:00	NO				9	02:00	NO				4	01:00	NO
WASH 5	TAP WATER	10	01:00	NO	6	01:00	NO												
8	ALCIAN BLUE																1	20:00	YES
9	LUGOL'S IODINE	1	05:00	YES							2	05:00	YES						
10	SOD. HYPOCHLORITE	3	02:00	YES															
11	PERIODIC ACID										1	05:00	YES						
12	IRON SOL / LUXOL FAST BLUE													1 (IRON SOL.)	10:00	YES			
13	SCHIFF'S REAGENT										4	10:00	NO						
14	1% ALCIAN YELLOW																		
15	CELESTINE BLUE																		
16	HARRIS' HAEMATOXYLIN	5	05:00	YES	1	05:00	YES				6	02:00	YES						
17	EOSIN	9	02:00	YES	5	02:00	YES												
18	GOMORI'S TRICHROME																		
EXIT	DISTILLED WATER	11			7			3			10			5			5		

Appendix 2. Spreadsheet to Determine Placement of Reagent Containers

		HAEM +	VAN GIESON		ALCIAN	YELLOW			SCHMORL		LUXOL	FAST BLUE		GOMORI	TRICHROME		ALCIAN BLUE	PRE PAS	
STATION	REAGENT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT	STEP	TIME	EXACT
ENTRY	DISTILLED WATER																		
OVEN @ 60 C	BOUIN'S SOL. / DIASTASE													1 (BOUIN'S)	30:00	YES			
1	1% NEUTRAL RED							4	05:00	YES									
2	1% ACETIC ACID							2	01:00	YES									
3	VAN GIESON	7	02:00	YES															
4	1% ACID ALCOHOL	5	00:10	YES										7	00:10	YES			
5	SCHMORL'S REAGENT							1	05:00	YES									
6	1% AQ. TOLUIDINE BLUE				7	03:00	YES												
7	DISTILLED WATER																		
WASH 1	TAP WATER				2	02:00	NO	3	05:00	NO	2	01:00	NO	2	05:00	NO			
WASH 2	TAP WATER	2	02:00	NO	4	02:00	NO	5	01:00	NO				4	02:00	NO			
WASH 3	TAP WATER	4	02:00	NO	6	02:00	NO							6	02:00	NO	2	02:00	NO
WASH 4	TAP WATER	6	10:00	NO	8	02:00	NO							8	10:00	NO			
WASH 5	TAP WATER	8	01:00	NO										10	01:00	NO			
8	ALCIAN BLUE																1	20:00	YES
9	LUGOL'S IODINE																		
10	SOD. HYPOCHLORITE				3	02:00	YES												
11	PERIODIC ACID				1	10:00	YES												
12	IRON SOL / LUXOL FAST BLUE										1 (LFB)	45:00	YES						
13	SCHIFF'S REAGENT																		
14	1% ALCIAN YELLOW				5	05:00	YES												
15	CELESTINE BLUE	1	05:00	YES										3	05:00	YES			
16	HARRIS' HAEMATOXYLIN	3	05:00	YES										5	05:00	YES			
17	EOSIN																		
18	GOMORI'S TRICHROME													9	20:00	YES			
EXIT	DISTILLED WATER	9			9			6			3			11			3		
					BLOT DRY	CLEAR AND	MOUNT				DIFFERENTIATE MANUALLY			BRIEFLY MANUALLY WASH				PUT BACK ON XL AT	
											THEN PUT BACK ON XL			IN 1% ACETIC ACID				PAS	
											AT H & E								

Appendix 3. Staining Programs 1-6

PROG. 1		H&E WITH IODINE PRE-TREATMENT		
STEP	TUB	REAGENT	TIME	EXACT
1	9	IODINE	5:00	YES
2	WASH 1	TAP WATER	2:00	NO
3	10	SOD. "HYPO"	2:00	YES
4	WASH 2	TAP WATER	2:00	NO
5	16	HEMATOXYLIN	5:00	YES
6	WASH 3	TAP WATER	2:00	NO
7	4	1% ACID ALCOHOL	0:10	YES
8	WASH 4	TAP WATER	5:00	NO
9	17	EOSIN	2:00	YES
10	WASH 5	TAP WATER	1:00	NO
11	EXIT	DIST. WATER	N.A	N.A

PROG. 2		DIASTASE BEFORE PAS		
STEP	TUB	REAGENT	TIME	EXACT
1	60°C OVEN	DIASTASE	20:00	YES
2	WASH 1	TAP WATER	3:00	NO
3	EXIT	DIST. WATER	N.A	N.A
4	PUT BACK ON			
5	AUTOSTAINER AT			
6	PROGRAM 4 FOR PAS			
7				
8				
9				
10				
11				

PROG. 3		GOMORI TRICHROME		
STEP	TUB	REAGENT	TIME	EXACT
1	60°C OVEN	BOUIN'S FLUID	30:00	YES
2	WASH 1	TAP WATER	5:00	NO
3	15	CELESTINE BLUE	5:00	YES
4	WASH 2	TAP WATER	2:00	NO
5	16	HEMATOXYLIN	5:00	YES
6	WASH 3	TAP WATER	2:00	NO
7	4	1% ACID ALCOHOL	0:10	YES
8	WASH 4	TAP WATER	10:00	NO
9	18	GOMORI'S STAIN	20:00	YES
10	WASH 5	TAP WATER	1:00	NO
11	EXIT	DIST. WATER	N.A	N.A

PROG. 4		PAS		
STEP	TUB	REAGENT	TIME	EXACT
1	11	PERIODIC ACID	5:00	YES
2	WASH 1	TAP WATER	2:00	NO
3	7	DIST. WATER	1:00	YES
4	13	SHIFF'S REAGENT	10:00	NO
5	WASH 2	TAP WATER	10:00	NO
6	16	HEMATOXYLIN	2:00	YES
7	WASH 3	TAP WATER	2:00	NO
8	4	1% ACID ALCOHOL	0:10	YES
9	WASH 4	TAP WATER	2:00	NO
10	EXIT	DIST. WATER	N.A	N.A

PROG. 5		ALCIAN BLUE PRE- PAS		
STEP	TUB	REAGENT	TIME	EXACT
1	8	ALCIAN BLUE	20:00	YES
2	WASH 3	TAP WATER	2:00	NO
3	EXIT	DIST. WATER	N.A	N.A
4	PUT BACK ON			
5	AUTOSTAINER AT			
6	PROGRAM 4 FOR PAS			
7				
8				
9				
10				

PROG. 6		ALCIAN BLUE		
STEP	TUB	REAGENT	TIME	EXACT
1	8	ALCIAN BLUE	20:00	YES
2	WASH 3	TAP WATER	2:00	NO
3	1	NEUTRAL RED	5:00	YES
4	WASH 4	TAP WATER	1:00	NO
5	EXIT	DIST. WATER	N.A	N.A
6				
7				
8				
9				
10				

Appendix 4. Staining Programs 7-12

PROG. 7		ALCIAN YELLOW		
STEP	TUB	REAGENT	TIME	EXACT
1	11	1% PERIODIC ACID	10:00	YES
2	WASH 1	TAP WATER	2:00	NO
3	10	SOD. "HYPO"	2:00	YES
4	WASH 2	TAP WATER	2:00	NO
5	14	1% ALCIAN YELLOW	5:00	YES
6	WASH 3	TAP WATER	2:00	NO
7	6	TOLUIDINE BLUE	3:00	YES
8	WASH 4	TAP WATER	2:00	NO
9	EXIT	DIST. WATER	N.A	N.A

PROG. 8		SCHMORL		
STEP	TUB	REAGENT	TIME	EXACT
1	5	SCHMORL'S SOL.	5:00	YES
2	2	1% ACETIC ACID	1:00	YES
3	WASH 1	TAP WATER	5:00	NO
4	1	1% NEUTRAL RED	5:00	YES
5	WASH 2	TAP WATER	1:00	NO
6	EXIT	DIST. WATER	N.A	N.A
7				
8				
9				

PROG. 9		PERL PRUSSIAN BLUE (IRON)		
STEP	TUB	REAGENT	TIME	EXACT
1	12	IRON SOLUTION	10:00	YES
2	WASH 1	TAP WATER	5:00	NO
3	1	1% NEUTRAL RED	5:00	YES
4	WASH 2	TAP WATER	1:00	NO
5	EXIT	DIST. WATER	N.A	N.A
6				
7				
8				
9				

PROG. 10		ROUTINE H&E		
STEP	TUB	REAGENT	TIME	EXACT
1	16	HEMATOXYLIN	5:00	YES
2	WASH 3	TAP WATER	2:00	NO
3	4	1% ACID ALCOHOL	0:10	YES
4	WASH 4	TAP WATER	5:00	NO
5	17	EOSIN	2:00	YES
6	WASH 5	TAP WATER		NO
7	EXIT	DIST. WATER	N.A	N.A
8				
9				

PROG. 11		LUXOL FAST BLUE		
STEP	TUB	REAGENT	TIME	EXACT
1	12	LUXOL FAST BLUE	45:00	YES
2	WASH 1	TAP WATER	1:00	NO
3	EXIT	DIST. WATER	N.A	N.A
4		DIFFERENTIATE		
5		MANUALLY THEN		
6		PUT BACK ON		
7		AUTOSTAINER		
8		AT PROGRAM 10 (H&E)		
9				

PROG. 12		HEMATOXYLIN & VAN GIESON		
STEP	TUB	REAGENT	TIME	EXACT
1	15	CELESTINE BLUE	5:00	YES
2	WASH 2	TAP WATER	2:00	NO
3	16	HEMATOXYLIN	5:00	YES
4	WASH 3	TAP WATER	2:00	NO
5	4	1% ACID ALCOHOL	0:10	YES
6	WASH 4	TAP WATER	10:00	NO
7	3	VAN GIESON	2:00	YES
8	WASH 5	TAP WATER	1:00	NO
9	EXIT	DIST. WATER	N.A	N.A