

Contents

Part I The Object

1 Introduction.....	3
References.....	9
2 Quality Control and Quality Assessment.....	11
Quality.....	11
Quality Control	12
Quality Assessment.....	12
Differential Features of Quality Control and Quality Assessment.....	13
Is 10% Random Review of Negative Pap Tests QC?	14
Total Quality Management	15
Analyzing Quality Control and Quality Assessment Activities	15
References.....	17
3 Specimen Collection.....	19
Nongynecologic Specimens.....	19
Fresh Body Cavity Fluids: To Clot or Not?	21
Gynecologic Specimens.....	23
Background	23
Conventional Pap Test	24
Liquid-Based Preparation	25
References.....	30

4 Salt Solutions	33
Historic Milestones	33
Normal Saline	34
Balanced Electrolyte Solutions	37
Balanced Salt Solutions	37
Isotonicity and Iso-osmolarity	38
References	39
5 Slide Preparation	41
Historic Milestones	41
Background	42
Perspective: Gyn Versus Non-gyn Specimens	42
Slide Preparation	44
Quality Control and Quality Assessment	46
Direct Smears	49
Adhesive “Aids”	49
Albuminized Slides	50
Frosted Slides	51
Charged Slides	52
Why Cells Don’t Stick to Glass	53
Making Glass Wettable	54
Fine Needle Aspiration Smears	56
Fine Needle Gage	58
Manual Liquid-Based Nongynecologic Preparations	59
Saccomanno Sputum Homogenization	59
Materials for Saccomanno’s Method	59
Method of Saccomanno (Modified)	60
Manual Liquid-Based Gynecologic Preparations ...	62
Automated Liquid-Based Preparations	62
Conclusion	65
Appendix A. Saponin Technique for Bloody	
Fresh Cell Suspensions	65
A.1 Materials	66
A.2 Method	67
A.3 Results	67
A.4 Discussion	68
References	68

6	Cytocentrifugation	73
	Historic Milestones	73
	Estimating Sample Size	76
	Don't Add More Specimen than Sample Chamber	
	Can Hold	79
	Retaining Cells on Slides	80
	Conclusion	82
	References.....	82
7	Membrane Filtration	85
	Historic Milestones	85
	Materials and Methods.....	87
	Materials	87
	Methods (Performed Within a Biological	
	Safety Cabinet)	88
	Results.....	89
	Discussion	89
	References.....	99
8	Fixation	101
	Historic Milestones	101
	Hierarchy of Fixation Materials and Methods	105
	Substitute Alcohols	105
	Air-Drying of Protected Fixed Cells.....	107
	Air-Drying and Rehydration of	
	Unprotected Cells	108
	Preservation.....	110
	Putting the Pieces of the Puzzle Together	110
	Cellular Water Content.....	112
	Cell Location When Preserved or Fixed.....	115
	Alcohol Chain Length.....	116
	Alcohol Concentration	117
	Whether Maintained Wet or Allowed to Air-Dry ..	118
	Location If and When Air-Dried.....	118
	Whether Carbowax Is Present When Air-Drying	
	Takes Place	120
	GYN and FNA	122
	NON-GYN	122
	Global Observations and Considerations.....	122
	Summary	127
	References.....	127

9 Cell Block Preparation	131
Historic Milestones	131
Thrombin-Clot Technique.....	132
Materials	133
Method	133
Alternative Cell Block Methods	135
Thermo Scientific No. 7401150	136
Improved Capture	138
Improved Presentation	138
Improved Consistency	138
Cell Blocks and Immunohistochemistry	138
Discussion	140
References.....	140
10 Papanicolaou Stain.....	143
Historic Milestones	143
Materials and Methods.....	150
Gill Hematoxylin.....	150
Gill Modified OG.....	151
Gill Modified EA	153
Scott's Tap Water Substitute	153
Notes	156
Results.....	160
Discussion	163
Hematoxylin.....	163
Differentiating Hematoxylin	164
Bluing Hematoxylin.....	165
Orange G.....	166
EA	167
Rinses	173
STAT-Pap: A 12-Step, 2-Min, 4-Color Papanicolaou Stain for Rapid Evaluation.....	175
Materials	175
Methods.....	175
Notes	176
Quality Assessment Using Buccal Smears	176
Destaining	181
Troubleshooting	183
References.....	188

11 Cross-Contamination Control	191
Historic Milestones	191
Papanicolaou on Cross-Contamination.....	192
CLIA '88 §493.1274 Standard: Cytology.....	194
How Did CLIA '88 Become “Cross-Contaminated”?...	194
Materials	196
Methods.....	199
Discussion	200
Floaters Happen	201
Conclusion and Recommendations	204
Don't Do Unnecessary Work	204
Do Necessary Work	204
References.....	205
12 H&E Stain.....	207
Historic Milestones	207
Hematoxylin.....	208
Eosin	208
Notes	211
Results.....	214
Conclusion	215
References.....	215
13 Romanowsky Stains	217
Historic Milestones	217
Advantages of Romanowsky-Type Stains in Routine Cytological Practice	219
References.....	224
14 Special Stains.....	227
Classification of Biological Stains by FDA and Certification of “Special Stains” by the Biological Stain Commission	228
Special Stains	232
Manual Versus Automation of Special Stains Protocol.....	241
Conclusion	242
References.....	242
Suggested Reading.....	243

Part II The Image

15 Clearing	247
Historic Milestones	247
Xylene Alternatives	248
Xylene	249
Immortalizing Xylene	251
References.....	256
16 Mounting Media	259
Historic Milestones	259
Source and Nature of Resin: Chemical Nature	264
Solubility in Aromatic Hydrocarbons and Concentrations Necessary to Give Viscosity Similar to that of 60% (w/v) Canada Balsam in Xylene	268
Solutions: Nature and Amount of Solvent and Resin by Weight	268
Drying Rate of Solution: Rapid, Medium, Slow with Time Limits Stated	269
Freedom From Air Aspiration	270
Index of Refraction of Mounting Solutions and of Solid Resin to 3 Decimals	271
Conclusion	276
References.....	277
17 Cover Glasses	279
Historic Milestones	279
Royal Microscopical Society Specification of a Standard Microscope Cover Glass	281
American Society for Testing and Materials Standard Specification E211	282
Tolerance of Microscope Objectives to Deviations from 0.17-mm-Thick Cover Glasses	283
Numerical Aperture Impact on Image Quality	285
Mounting Medium Thickness	286
Unitized Pricing	287
Cover Glass Dimensions.....	289
Conclusion	290
References.....	291

18 Mounting	293
Historic Milestones	293
Coverslipping Millipore Filters.....	294
Materials for Halving 47-mm Millipore Filters.....	294
Method	295
Results	298
Discussion	299
Brown Artifact (aka “Cornflaking”)	299
Mounting Medium Thickness.....	303
Evaporative Weight Loss of Mounting Medium Solvent.....	303
“Cooking” Slides Reduces Mounting Medium Thickness and Hastens Hardening	305
References.....	307
19 Köhler Illumination	309
Cleaning the Microscope	312
Do: Tips.....	314
Do: Techniques	315
Eyepieces	315
Substage Condenser Top Lens.....	315
Objectives	315
Do: Timing.....	316
Daily	316
Weekly	316
Do: As Needed.....	316
Don’t	316
Practical Microscopy (aka “Glass-and-Brass” Tacks)	317
Working Köhler illumination.....	317
Light show	318
Cleanliness Is Next to Goodliness	318
Thickness of Slide.....	319
Working Numerical Aperture Salvages Image Quality	320
Depth-of-Field Versus Depth-of-Focus	321
Magnification Versus Enlargement and the “×” Factor	321
Photomicrograph Versus Microphotograph.....	322
A Breath of Fresh Air	322
References.....	323

Part III Everything Else

20 Screening	327
SPADE: Screening Protocol to Assist Detection	331
Preview	331
Examine	333
Review	334
The 4× Objective.....	334
Ink Dots.....	336
Percent Overlap.....	337
Gill Screening Reticle	340
Eyepiece Field Number.....	343
Conspicuity Area	345
Vigilance and the Vigilance Decrement.....	347
Screening: Search and Attention—Misunderstood and Undervalued.....	350
Conclusion	353
References.....	355
21 Bethesda System 2001, CLIA '88, and Data Analysis	359
Historic Milestones	359
Cellular Adequacy	364
Definitions of Screening and Screening Time	366
Workload Calculations.....	366
How Laboratorians Can Safely Calculate Workload for FDA-Approved Semi-automated Gynecologic Cytology Screening Devices	367
What Are the Current Issues with Workload Recording and Maximum Workload Limits?	367
How Can Laboratorians Safely Calculate Workload for FDA-Approved Semi-automated Cytology Screening Device [sic]?	367
Workload Limits	368
Evaluating a Cytotechnologist's Rescreening Errors with that of the Laboratory	369
False Negative Proportion.....	370

Calculating FNP (Estimated)	372
100% Rapid Review Versus 10% Slow Review (i.e, CLIA '88 10% Review).....	375
Conclusion	375
References.....	377
Appendix A: Word Notes	379
References.....	382
Appendix B: Arithmetic in the Cytopreparatory	
Laboratory	383
Percent Concentrations	384
Common Examples of Relative Concentration Expressions	384
Alcohols	385
Biological Dyes.....	385
Molarity.....	386
Normality	386
Acid Safety Notes	388
Temperature Conversion	388
Centrifugal Force	389
Formaldehyde Versus Formalin	390
Appendix C: Standard Precautions	391
Historical Milestone.....	392
References.....	392
Appendix D: Cell Transfer Technique	393
Purpose and Function.....	393
Materials	393
Method	394
References.....	395
Appendix E: Lagniappe	397
Appendix F: Use of the Word “Chromatin”	401
References.....	402

Appendix G: Useful URL	403
Appendix H: Selected Milestones in Microtechnique.....	407
Reference	409
Appendix I: Screening and CPR	411
Reference	414
Appendix J: Author's Awards and Publications.....	415
Notes	415
Awards and Honors	415
Publications (269)	416
Books	416
Book Chapters.....	417
Peer-Reviewed Articles.....	418
Abstracts	419
Articles.....	421
Letters to the Editor	424
Vendor Literature	425
Index.....	427