6TH EDITION

DIAGNOSTIC MEDICAL PARASITOLOGY
6TH EDITION

DIAGNOSTIC MEDICAL PARASITOLOGY

LYNNE SHORE GARCIA, M.S., MT, CLS, F(AAM)
LSG & Associates, Santa Monica, California

Washington, DC
Dedication

As with the first five editions, I dedicate this book to Marietta Voge, a truly rare individual who was widely recognized as one of the world's leading parasitologists. During her years as a diagnostic and research parasitologist at the University of California, Los Angeles, she touched the lives of many students and staff in a very special way. She was always more than willing to share her expertise with all who asked and volunteered this help over the years whenever contacted. She was always willing to donate a considerable amount of her personal time as a volunteer for various medical projects throughout the world.

She was a very special individual to work with, always interested in the person as well as the problem at hand. Her areas of teaching extended far beyond science. Whatever subject she was interested in received her total enthusiasm and dedication, and she had an exceptional ability to deal with detailed work. Her sense of fairness and professional integrity were remarkable; these ideals were shared with all who came in contact with her.

Her contributions to the field of diagnostic parasitology were numerous and included many classes, seminars, papers, and textbooks. The importance of working with Dr. Voge is hard to put into words. She was unique in her ability to allow a student to grow, both scientifically and personally. She could guide without constraints, teach without formal lectures, counsel without being judgmental, challenge without being unrealistic, tease without being cruel, and always be supportive regardless of the situation. She expected much from her students and employees and yet always gave considerably more than she received.

Scientific information gained from our association with her was invaluable; however, her impact on our lives was considerably more than scientific. She was always available for consultations and just to talk. She left all of us with a sense of having personally matured as a result of knowing and working with her over the years. She is missed by all of us, and yet her contributions in terms of teaching, consultations, volunteer work, professionalism, and friendship will remain with us forever.

I would also like to dedicate the sixth edition of this book to the bench technologists, those of you who provide critical diagnostic information on a daily basis and contribute such valuable input for excellent patient care.
Academic training provides key information in the field, but those who perform routine work at the bench often contribute much more than simple diagnostic identifications. Congratulations and thanks to all of you.

Finally, I also dedicate this book to John Lawrence. He was an extraordinary individual, and without his original encouragement and assistance, the first edition of the book would never have been written.
Contents

Dedication v
Preface xi
Acknowledgments xv

PART I
Diagnostic Procedures 1

1 Philosophy and Approach to Diagnostic Parasitology 3

2 Collection, Preservation, and Shipment of Fecal Specimens 6
Safety 6
Fresh-specimen collection 7
Collection of the specimen 7
Number of specimens to be collected (standard recommendation) 7
Number of specimens to be collected (pros and cons of various options) 8
Collection times 9
Specimen type, specimen stability, and need for preservation 9
Preservation of specimens 12
Preservatives 12
Use of fixatives 20
Shipment of diagnostic specimens, biological products, etiologic agents, or infectious substances 21

3 Macroscopic and Microscopic Examination of Fecal Specimens 26
Macroscopic Examination 26
Microscopic Examination (Ova and Parasite Examination) 27
Direct wet smear 27
Concentration (sedimentation and flotation) 32
Permanent stained smear 41

Specialized Stains for Coccidia (Cryptosporidium, Cystoisospora, and Cyclospora Species) and the Microsporidia 60
Modified Kinyoun’s acid-fast stain (cold method) 60
Modified Ziehl-Neelsen acid-fast stain (hot method) 63
Carbol fuchsin negative stain for Cryptosporidium (from W. L. Current) 66
Rapid safranin method for Cryptosporidium 66
Rapid safranin method for Cyclospora, using a microwave oven 66
Auramine O stain for coccidia (from Thomas Hänscheid) 67
Modified trichrome stain for the microsporidia (Weber—green) 68
Modified trichrome stain for the microsporidia (Ryan—blue) 70
Modified trichrome stain for the microsporidia (Kokoskin—hot method) 72
Acid-fast trichrome stain for Cryptosporidium and the microsporidia 72

4 Additional Techniques for Stool Examination 77
Culture of larval-stage nematodes 77
Harada-Mori filter paper strip culture 78
Filter paper/slant culture technique (petri dish) 79
Charcoal culture 80
Baermann technique 81
Agar plate culture for Strongyloides stercoralis 83

Egg studies 87
Estimation of worm burdens and Kato-Katz thick film 87
Hatching of schistosome eggs 89
Search for tapeworm scolex 91
India ink injection procedure for tapeworm proglottids 92
Qualitative test for fecal fat 94
Quantitation of reducing substances (Clinitest) 95
5 Examination of Other Specimens from the Intestinal Tract and the Urogenital System 98

Examination for pinworm 98
Cellulose tape preparations 99
Anal swabs 99

Sigmoidoscopy material 100
Direct saline mount 101
Permanent stained slide 101
Duodenal contents 102
Duodenal drainage 102
Duodenal capsule technique (Entero-Test) 103

Urogenital specimens 103
Trichomoniasis 103
Filariasis 105
Schistosomiasis 105

6 Sputum, Aspirates, and Biopsy Material 107

Expectorated sputum 107
Induced sputum 109

Aspirates 116
Lungs and liver 116
Lymph nodes, spleen, liver, bone marrow, spinal fluid, eyes, and nasopharynx 118
Cutaneous ulcer 120

Biopsy material 120
Skin 124
Lymph nodes 124
Muscle 125
Rectum and bladder 126

7 Procedures for Detecting Blood Parasites 129

Preparation of thick and thin blood films 129
Thick blood films 130
Thin blood films 131
Combination thick and thin blood films (on the same slide) 132
Combination thick and thin blood films (can be stained as either) 132
Buffy coat blood films 134

Staining blood films 135
Giemsa stain 136
Wright's stain 137
General notes on staining procedures 140

Proper examination of thin and thick blood films 140
Thin blood films 140
Thick blood films 141
Determination of parasitemia 141

Diagnosis of malaria: review of alternatives to conventional microscopy 142
QBC microhematocrit centrifugation method 145
Parasight F test 146
NOW malaria test 147

Flow anti-pLDH Plasmodium monoclonal antibodies 148
Molecular testing 149
Automated blood cell analyzers 150

Diagnosis of leishmaniasis: review of alternatives to conventional microscopy 150
ICT for detection of anti-rK-39 antibodies 150
Concentration procedures 151
Cytocentrifugation technique 151
Knott concentration procedure 151
Membrane filtration technique 151
Gradient centrifugation technique 152

Triple-centrifugation method for trypanosomes 152
Special stain for microfilarial sheath 152
Delafield's hematoxylin 152

8 Parasite Recovery: Culture Methods, Animal Inoculation, and Xenodiagnosis 156

Culture methods 156
Intestinal protozoa 157
Pathogenic free-living amebae 162
Blastocystis spp. (Blastocystis hominis) 167
Pathogenic flagellates 168
Flagellates of blood and tissue 172
Toxoplasma gondii 176
Plasmodium and Babesia spp. 177
Cryptosporidium spp. 178
Microsporidia 178

Animal inoculation 178
Leishmania spp. 180
Trypanosoma spp. 180
Toxoplasma gondii 180

Xenodiagnosis 181

9 Fixation and Special Preparation of Fecal Parasite Specimens and Arthropods 183

Fixation of parasite specimens and arthropods 183
Protozoa 185
Solutions to induce relaxation in adult helminths 185
Nematodes 186
Trematodes 187
Cestodes 187
Helminth eggs and larvae 188
Arthropods 188

Mounting and staining of parasite specimens for examination 189
Nematodes 189
Trematodes 189
Cestodes 191

Mounting of arthropods for examination 191
Mites 192
Fleas and lice 192
Ticks 193
Miscellaneous arthropods 193
## Contents

### 10 Artifacts That Can Be Confused with Parasitic Organisms

**Protozoa**
- Amebae 195
- Flagellates 198
- Ciliates 198
- Coccidia and microsporidia 198
  - *Cryptosporidium* spp. and *Cyclospora cayetanensis* 198
  - *Cryptosporidium bellii* 198
  - *Microsporidia* 199

**Blood and body fluids**
- Malaria parasites and *Babesia* spp. 200
- Leishmanias and trypanosomes 200
- Microfilariae 201
- Body fluids: ciliated epithelial cells 202

**Helminths**
- Adult worms and larvae 203
- Eggs 204

**Human cells**
- Polymorphonuclear leukocytes 205
- Eosinophils 206
- Macrophages 207
- Lymphocytes 207
- Red blood cells 207
- Charcot-Leyden crystals 208

**Nonhuman elements seen in feces (yeast cells)** 209

**Insect larvae** 209

**Spurious infections** 209

**Delusory parasitosis (delusional infestation)** 210

### 11 Equipment, Supplies, Safety, and Quality System Recommendations for a Diagnostic Parasitology Laboratory: Factors Influencing Future Laboratory Practice

**Equipment**
- Microscope 212
- Centrifuge 216
- Fume hood 217
- Biological safety cabinet 217
- Refrigerator-freezer 218

**Supplies**
- Glassware 218
- Miscellaneous supplies 218
- ATCC quality control organisms 219

**Safety: personnel and physical facilities**
- General precautions 219
- Handwashing 220
- Personal protective equipment (OSHA 2001 blood borne) 221
- Handling specimens 221
- Processing specimens 221
- Spills 222

- Disposal of contaminated materials 224
- Standard precautions 224
- Hepatitis exposure protocol 227
- Dangerous properties of industrial materials 227
- Current OSHA regulations for the use of formaldehyde 228
- Latex allergy 229

**Quality systems**
- Extent of services 229
- Proficiency testing 230
- In-house quality control 233
- Patient outcome measures 236
- Continuous quality improvement, total quality management, or 10-step and FOCUS-PDCA for performance improvement activities 237
- CLIA '88 inspection process 238

**New quality guidelines**
- ISO guidelines 240
- CLSI (NCCLS) model 240

**Factors influencing future laboratory practice**
- Managed care 241
- Financial considerations 242
- Current regulations 242
- Decentralized testing 243
- Laboratory services 244
- Technological trends 244
- Clinical decision support 245
- Personnel issues 245
- Changing demographics 245
- Emerging diseases 246
- Bioterrorism 246

### 12 Medical Parasitology: Case Histories

**Protozoal infections**
- Case 1 249
- Case 2 251
- Case 3 252
- Case 4 255
- Case 5 256
- Case 6 258
- Case 7 260

**Helminth infections**
- Case 8 262
- Case 9 263
- Case 10 266
- Case 11 267
- Case 12 269
- Case 13 271
- Case 14 273

**Blood parasite infections**
- Case 15 274
- Case 16 278
- Case 17 280
Contents

Case 18 281
Case 19 283
Case 20 284
Diagnostic methods and proficiency testing 286
Case 21 286
Case 22 288
Case 23 289
Case 24 292
Case 25 293

PART II
Clinically Important Human Parasites 297

13 Intestinal Nematodes 299
Ascaris lumbricoides 300
Enterobius vermicularis 308
Trichuris trichiura 311
Capillaria philippinensis 314
Hookworms (Ancylostoma duodenale, Necator americanus, and Ancylostoma ceylanicum) 316
Trichostrongylus spp. 321
Strongyloides spp. 322

14 Tissue Nematodes 336
Trichinella spp. 336
Baylisascaris procyonis 346
Lagochilascaris minor 352
Toxocara canis and T. cati (visceral larva migrans and ocular larva migrans) 353
Ancylostoma braziliense and A. caninum (cutaneous larva migrans) 358
Human eosinophilic enteritis 359
Dracunculus medinensis 360
Angiostrongylus (Parastrongylus) cantonensis (cerebral angiostrongylitis) 362
Angiostrongylus (Parastrongylus) costaricensis (abdominal angiostrongylitis) 365
Gnathostoma spinigerum 366
Gnathostoma doloresi, G. nipponicum, G. hispidum, and G. binucleatum 368
Anisakis simplex, A. physetis, Pseudoterranova decipiens, Contracecum osculatum, Hysterobothrium adenuncum, and Porrocaecum reticulatum (larval nematodes acquired from saltwater fish) 370
Capillaria hepatica 373
Thelazia spp. 373

15 Filarial Nematodes 377
Basic Life Cycle 380
The Endosymbiont 380
Human Pathogens 380
Wuchereria bancrofti 381
Brugia malayi 391
Brugia timori 392
Zoonotic Brugia infections (American brugian filariasis) 393
Tropical pulmonary eosinophilia 394
Loa loa 394
Mansonella ozzardi 397
Mansonella perstans 398
Mansonella streptocerca 399
Onchocerca volvulus 400
Dirofilaria andes, Dirofilaria andes and Dirofilaria Nochtiella spp. 409

16 Intestinal Cestodes 418
Diphyllobothrium latum 418
Taenia solium 425
Taenia saginata 435
Taenia asiatica (Asian Taenia or Taenia saginata asiatica) 437
Hymenolepis (Rodentolepis) nana 439
Hymenolepis diminuta 441
Dipylidium caninum 443

17 Tissue Cestodes: Larval Forms 447
Echinococcus granulosus (cystic hydatid disease) 447
Echinococcus multilocularis (alveolar disease, hydatid disease) 458
Echinococcus vogeli (Polycystic Hydatid Disease) and Echinococcus oligarthrus (Unicystic Hydatid Disease): Neotropical Echinococcosis 463
Taenia (Multiceps) spp. (Taenia multiceps, Taenia serialis) (coenurosis) 465
Spirometra mansonioides and Diphyllobothrium spp. (sparganosis) 467

18 Intestinal Trematodes 474
Fasciolopsis buski 475
Echinostoma ilocanum 479
Heterophyes heterophyes 481
Metagonimus yokogaei 482
Gastrodiscoides bosminii 484

19 Liver and Lung Trematodes 487
Liver Flukes 487
Clonorchis sinensis 487
Opisthorchis viverrini 494
Opisthorchis felineus 497
Fasciola hepatica 499
Fasciola gigantica 502
Less Common Liver Flukes 504
Dicrocoelium dendriticum, Dicrocoelium hospes, and Eurytrema pancreaticum 504
Lung Flukes 506
Paragonimus westermani 506
Paragonimus kellicotti 512

20 Blood Trematodes: Schistosomes 516
Schistosoma mansoni 517
Schistosoma japonicum 530
Schistosoma mekongi 536

11/24/15 9:51 PM
Contents

21 Intestinal Protozoa: Amebae 552

Entamoeba histolytica 552
Entamoeba dispar 567
Entamoeba moshkovskii 568
Entamoeba bangledeshi 569
Entamoeba hartmanni 570
Entamoeba coli 571
Entamoeba polecki 572
Entamoeba gingivalis 572
Endolimax nana 574
Iodamoeba bütschlii 575
Blastocystis spp. 576

22 Intestinal Protozoa: Flagellates and Ciliates 584

Giardia lamblia (G. duodenalis, G. intestinalis) 584
Dientamoeba fragilis 598
Pentatrichomonas hominis (Trichomonas hominis) 602
Trichomonas tenax 603
Chilomastix mesnili 604
Enteromonas hominis 604
Retortamonas intestinalis 605
Balantidium coli 605

23 Intestinal Protozoa (Coccidia), Microsporidia, and Algae 612

Coccidia 612
Cryptosporidium spp. 612
Cyclospora cayetanensis 630
Cystoisospora (Isospora) belli 637
Sarcocystis spp. 643
Microsporidia 648
Algae (Prototheca) 662

24 Free-Living Amebae 667

Naegleria fowleri 669
Acanthamoeba spp. 677
Balamuthia mandrillaris 687
Sappinia (diploidea) pedata 690

25 Protozoa from Other Body Sites 694

Trichomonas vaginalis 694
Toxoplasma gondii 704

26 Malaria and Babesiosis 719

Malaria 719
Babesiosis 763

27 Leishmaniasis 778

Cutaneous Leishmaniasis: General Comments 779
Old World (Eastern) Leishmaniasis: Cutaneous Leishmaniasis 780
New World (Western) Leishmaniasis: Cutaneous Leishmaniasis 788
Visceral Leishmaniasis: General Comments 796
Old World (Eastern) Leishmaniasis: Visceral Leishmaniasis 796
New World (Western) Leishmaniasis: Visceral Leishmaniasis 803

28 Trypanosomiasis 810

African trypanosomiasis 811
Trypanosoma brucei gambiense 811
Trypanosoma brucei rhodesiense 822
American trypanosomiasis 826
Trypanosoma cruzi 826
Trypanosoma rangeli 839

29 Unusual Parasitic Infections 845

Aquatic protist 845
Rhinosporidium seeberi 845
Protozoa 848
Lophomonas blattarum 848
Dictyostelium polycephalum 850
Myxozoa parasites 850
Trypanosoma evansi, Trypanosoma lewisi 851
Nematodes (the roundworms) 852
Anclylostoma ceylanicum 852
Halicephalobus gingivalis 853
Oesophagostomum spp. 853
Eustrongylides spp. 855
Mermis nigrescens 856
Dictyophyma renale 856
Ternidens deminutus 858
Mammomonogamus laryngeus (Syngamus laryngeus) 859
Ascaris suum 860
Gongylonema pulchrum 861
Haycocknema perplexum 861
Cestodes 862
Diplogonoporus spp. 862
Bertiella studeri 862
Inermicapsifer madagascariensis 863
Raillietina celebensis 863
Mesocestoides spp. 864
Taenia crassiceps 865
Trematodes 866
Alaria americana 866
Plagiorchis spp. 868
Neodiplostomum seoulense 868
Spelotrema brevicaca 869
Brachylaima sp. 869
Nanophyetus (Troglotrema) salmincola 870
Stellantchasmus falcatus 871
Phaneropsolus spinicirrus, Phaneropsolus bonni, and Prosthodendrium molenkempi 871
Contents

Haplorchis taichui 872
Gymnophalloides seoi 872
Metorchis conjunctus (North American liver fluke) 873
Schistosoma mattheei 874
Philophthalmus lacrinosus 875
Achillurbainia spp. 875

Pentastomids 875
Armillifer spp., Linguatula serrata, and Sebekia spp. 875

Acanthocephalans 876
Macracanthorhynchus hirudinaceus and Moniliformis moniliformis 876

30 Parasitic Infections in the Compromised Host 883
Entamoeba histolytica 885
Free-living amebae 895
Blastocystis spp. 903
Giardia lamblia 903
Toxoplasma gondii 905
Cryptosporidium spp. 907
Cyclospora cayetanensis 910
Cystoisospora (Isospora) belli 912
Sarcocystis spp. 913
Microsporidia 914
Leishmania spp. 918
Strongyloides stercoralis 923
Plasmodium spp. 926
Babesia spp. 927
American trypanosomiasis 929
Crusted scabies 930

31 Health Care-Associated and Laboratory-Acquired Infections 935
Health Care-Associated (Nosocomial) infections 935
Gastrointestinal infections 936
Cryptosporidium spp. 936
Giardia lamblia 939
Entamoeba histolytica 940
Microsporidia 941
Cystoisospora (Isospora) belli 941
Hymenolepis nana 942
Taenia solium 942
Blood and tissue infections 942
Plasmodium spp. 942
Babesia spp. 943
Trypanosoma brucei gambiense and T. brucei rhodesiense 943
Trypanosoma cruzi 943
Leishmania donovani 944
Toxoplasma gondii 944
Infections with ectoparasites 945
Pediculus spp. and Phthirus pubis 945
Sarcoptes scabiei 945
Myiasis 945

Infections in the pediatric patient 946
Cryptosporidium spp. 946
Giardia lamblia 946
Pediculus humanus capitis 946
Sarcoptes scabiei 946
Infections in the compromised patient 946
Laboratory infections 948
Intestinal protozoa 948
Free-living amebae 948
Plasmodium spp. 948
Trypanosoma brucei gambiense and T. brucei rhodesiense 948
Trypanosoma cruzi 950
Leishmania spp. 950
Toxoplasma gondii 951
Specimen handling 951
Summary 951

32 Immunology of Parasitic Infections 954
Amebiasis 960
Giardiasis 964
Toxoplasmosis 966
African trypanosomiasis 968
American trypanosomiasis 970
Malaria 973
Helminth infections 978
Summary 981

33 Antibody and Antigen Detection in Parasitic Infections 986
Protozoal infections 993
Amebiasis 993
Babesiosis 995
Chagas' disease 996
Cryptosporidiosis 997
Cyclosporiasis 997
Giardiasis 997
Leishmaniasis 998
Malaria 999
Toxoplasmosis 1001
Trichomoniasis 1004
Helminth infections 1004
Cysticercosis 1004
Hydatid disease 1005
Fascioliasis 1007
Filariasis 1007
Paragonimiasis 1009
Schistosomiasis 1010
Strongyloidiasis 1011
Toxocarasis 1012
Trichinosis 1013
Intradermal tests 1014
Casoni test 1014
Montenegro test 1014
34 Histologic Identification of Parasites 1019

Protozoa 1020
Amebas 1020
Flagellates 1024
Ciliates 1024
Coccidia 1025
Microsporidia 1027
Helminths 1029
Nematodes 1029
Cestodes 1039
Trematodes 1045
Blood Parasites 1052
Malaria 1052
Leishmania 1053
Trypanosomes 1055
Filaria 1056

35 Medically Important Arthropods 1077

Arthropods and their relationship to disease 1077
Biological vectors of microorganisms 1077
Bites and envenomation 1078
Tissue invasion 1081
Entomophobia and delusional infestation (parasitosis) 1082

Class Insecta (insects) 1083
Order Diptera (flies, mosquitoes, and midges) 1083
Myiasis 1090
Order Hemiptera (true bugs) 1098
Order Coleoptera (beetles) 1100
Order Siphonaptera (Fleas) 1108
Order Anoplura (sucking lice) 1104
Order Mallophaga (biting and chewing lice) 1106
Order Hymenoptera (bees, wasps, and ants) 1106
Order Blattaria (cockroaches) 1108

Class Arachnida (ticks, mites, spiders, and scorpions) 1109
Subclass Acari (ticks, mites, and chiggers) 1109
Subclass Aranae (spiders) 1118
Subclass Scorpiones (scorpions) 1122

Other arthropods 1122
Class Chilopoda (centipedes) 1122
Class Diplopoda (millipedes) 1123
Class Crustacea (copepods, crabs, crayfish, etc.) 1123

Control of arthropods of medical importance 1123
Physical control 1124
Biological control 1124
Chemical control 1124

36 Treatment of Parasitic Infections 1134

Albendazole (Albenza) (Amedra) 1134
Amphotericin B (AmBisome) (Gilead) 1135
Amphotericin B (Fungizone) (X-Gen) 1153
Artemether (Artemam) (Arenco, Belgium) 1154
Atovaquone (Mepron) (GlaxoSmithKline) 1154
Atovaquone-Proguanil (Malarone) (GlaxoSmithKline) 1155
Benznidazole (Rochagan) (Roche, Brazil) 1155
Bithionol (Bitin) (CDC) 1156
Chloroquine Phosphate (Aralen) (Sanofi, Others) 1156
Crotamiton (Eurax) (Ranbaxy) 1156
Dapsone (Jacobs) 1157
Diethylcarbamazine Citrate USP (Hetrazan) (CDC) 1157
Diloxanide Furoate (Furamid, Entamide) (Boots, England) 1157
Eflornithine (Difluoromethylornithine, Ornidyil) (Sanofi) (CDC) 1158
Fumagillin (Fumidil-B) 1158
Furazolidone (Furoxone) 1158
Iodoquinol/Diiodohydroxyquin (Yodoxin) (Glenwood, Others) 1158
Ivermectin (Stromectol, Sklice) (Merck, Sanofi) 1159
Lumefantrine/Artemether (Coartem, Riamet) (Novartis) 1160
Malathion (Ovide) (Taro Pharmaceuticals) 1160
Mebendazole (Generics) 1160
Mefloquine Hydrochloride (Generics) 1161
Melarsoprol (Mel-B) (CDC) 1162
Metronidazole (Flagyl, IV Flagyl) (Searle/Pfizer, Baxter) 1162
Miltefosine (Impavid, Miltex) (Paladin, Canada) (CDC) 1163
Niclosamide (Yomesan, Niclocide) (Bayer, Germany) 1163
Nifurtimox (Lampit) (Bayer HealthCare) (CDC) 1163
Nitazoxanide (Alinia) (Romark) 1164
Pentamidine Isethionate (Pentam 300, Nebupent) (APP Pharmaceuticals) 1165
Permethrin (Nix [Insight Pharmaceuticals], Elimite [Prestium Pharma]) 1166
Polyhexamethylene Biguanide (Baquacil) (Zenece) 1166
Praziquantel (Biltricide) (Bayer) 1166
Primaquine Phosphate (Sanofi-Aventis) 1167
Propamidine Isethionate (Brolene) (Aventis, Canada) 1167
Pyrantel Pamoate (Pin-X, Reese’s Pinworm Medicine) (Quartz Specialty Pharmaceuticals, Reese) 1168
Pyrethrin with Piperonyl Butoxide (Rid) (Bayer, Others) 1168
Pyrimethamine (Daraprim) (Amedra) 1168
Quinidine Gluconate (Generics) 1169
Quinine Sulfate or Quinine Dihydrochloride (Many Manufacturers) 1169
Sibogluconate Sodium (Pentostam, Solustibosan) (GlaxoSmithKline) (CDC) 1170
Suramin Sodium (Germanin) (Bayer, Germany) (CDC) 1170
Thiabendazole (Mintezol) (Merck) 1170
Tinidazole (Tindamax) (Mission Pharmaceuticals) 1171
Triclabendazole (Egaten) (Novartis) 1172

APPENDIXES 1175

APPENDIX 1  Information Tables 1176
A1.1 Classification of human parasites 1176
A1.2 Distribution of selected parasitic infections in the Americas 1179
A1.3 Distribution of selected parasitic infections in Europe 1179
A1.4 Distribution of selected parasitic infections in Africa 1180
A1.5 Distribution of selected parasitic infections in Asia 1180
A1.6 Distribution of selected parasitic infections in Oceania 1181
A1.7 Cosmopolitan distribution of common parasitic infections (North America, Mexico, Central America, South America, Europe, Africa, Asia, and Oceania) 1181
A1.8 Body sites and specimen collection 1182
A1.9 Body sites and possible parasites recovered (trophozoites, cysts, oocysts, spores, adults, larvae, eggs, amastigotes, and trypomastigotes) 1183
A1.10 Body site, specimen and procedures, recommended methods, relevant parasites, and comments 1184
A1.11 Examination of tissue and body fluids 1189
A1.12 Key characteristics of protozoa of the intestinal tract and urogenital system 1191
A1.13 Key characteristics of tissue protozoa 1194
A1.14 Key characteristics of helminths 1196
A1.15 Key characteristics of most common parasites found in blood 1198
A1.16 Diagnostic laboratory report information that should be relayed to the physician 1200
A1.17 Pros and cons of stool specimen collection and testing options 1201
A1.18 Approaches to stool parasitology: test ordering 1203
A1.19 Pros and cons of ova and parasite examination options 1204
A1.20 Laboratory test reports: optional comments 1206
A1.21 Estimated prevalence of parasitic diseases worldwide 1207

APPENDIX 2  Flowcharts and Staining Tables for Diagnostic Procedures 1208

Flowcharts
A2.1 Procedure for processing fresh stool for the ova and parasite examination 1208
A2.2 Procedure for processing liquid specimens for the ova and parasite examination 1209
A2.3 Procedure for processing preserved stool for the ova and parasite examination by using the traditional two-vial collection kit 1210
A2.4 Procedure for processing sodium acetate-acetic acid-formalin (SAF)-preserved stool for the ova and parasite examination 1211
A2.5 Use of various fixatives and their recommended stains: fecal specimens preserved using polyvinyl alcohol (PVA) 1212
A2.6 Use of various fixatives and their recommended stains: fecal specimens preserved in the Universal Fixative, TOTAL-FIX 1213

Tables
A2.1 Steps in the trichrome staining procedure (mercuric chloride-based PVA-preserved stool specimens) 1214
A2.2 Steps in the trichrome staining procedure (non-mercuric chloride-based PVA-preserved stool specimens) 1215
A2.3 Steps in the iron hematoxylin staining procedure (mercuric chloride-based PVA-preserved stool specimens) (Spencer-Monroe method) 1216
A2.4 Steps in the iron hematoxylin staining procedure (mercuric chloride-based PVA-preserved stool specimens) (Tompkins-Miller method) 1217
A2.5 Steps in the iron hematoxylin staining procedure (incorporating the carbol fuchsin step) 1218
A2.6 Steps in the trichrome staining procedure (Universal Fixative [no mercury, no formalin, no PVA]) 1219
A2.7 Oil-mounted permanent stained smears (no Permount is used) 1220
A2.8 Tips on stool processing and staining 1221

APPENDIX 3  Common Problems in Parasite Identification 1222

Figures
A3.1–A3.25 Paired drawings of “look alikes” 1222
A3.26 Relative sizes of helminth eggs 1233

Tables
A3.1 Entamoeba spp. trophozoites versus macrophages 1223
A3.2 Entamoeba spp. cysts versus polymorphonuclear leukocytes (PMNs) 1224
A3.3 Entamoeba bistolyctica versus Entamoeba coli precysts and cysts 1226
A3.4 Endolimax nana versus Dientamoeba fragilis 1227
A3.5 Adult nematodes and/or larvae found in stool specimens: size comparisons 1234

APPENDIX 4  Quality Control Recording Sheets 1235

A4.1 Diagnostic parasitology quality control (QC) (reagents) 1236
A4.2 Diagnostic parasitology quality control (QC) (reagents)—example for multiple reagents  1237
A4.3 Diagnostic parasitology quality control (QC) (culture)—example of a worksheet  1238
A4.4 Equipment maintenance  1239

APPENDIX 5  Commercial Supplies and Suppliers  1241

Tables
A5.1 Sources of commercial reagents and supplies  1242
A5.2 Addresses of suppliers listed in Table A5.1  1245
A5.3 Sources of available reagents for immunodetection of parasitic organisms or antigens  1246
A5.4 Addresses of suppliers listed in Table A5.3  1248
A5.5 Commercial suppliers of diagnostic parasitology products  1249
A5.6 Sources of additional teaching materials, including case histories  1252

A5.7 Sources of parasitologic specimens  1253

APPENDIX 6  Reference Sources  1254

APPENDIX 7  “Late-Breaking” Published Information  1257

APPENDIX 8  Molecular Panels for Parasitology  1278

APPENDIX 9  FAQs  1284


GLOSSARY  1307

Index  1321
During the past few years, the field of diagnostic medical parasitology has seen dramatic changes, including newly recognized parasites, emerging pathogens in new geographic areas, bioterrorism considerations and requirements, alternative techniques required by new regulatory requirements, reevaluation of diagnostic test options and ordering algorithms, continuing changes in the laboratory test menus, implementation of testing based on molecular techniques, reporting formats and report comments, coding and billing requirements, managed-care relevancy, increased need for consultation and educational initiatives for clients, and an overall increased awareness of parasitic infections from a worldwide perspective. We have seen organisms like the microsporidia change from the status of “unusual parasitic infection” to being widely recognized as among the most important infections in both immunocompetent and compromised patients. With confirmation of the fifth human malaria, Plasmodium knowlesi, this field has expanded dramatically. More sensitive diagnostic methods for organism detection in stool specimens are now commercially available for Entamoeba histolytica, Entamoeba dispar, Giardia lamblia, Cryptosporidium spp., and Trichomonas vaginalis. Reagents are actively being developed for other organisms such as Dientamoeba fragilis, Blastocystis spp., and the microsporidia. We have seen Cyclospora cayetanensis coccidia become well recognized as the cause of diarrhea in immunocompetent and immunocompromised humans. We continue to see new disease presentations in compromised patients; a good example is granulomatous amebic encephalitis caused by Acanthamoeba spp., Sappinia diploidea, and Balamuthia mandrillaris. With the expansion of transplantation options, many parasites are potential threats to patients who are undergoing immunosuppression, and these must be considered within the context of this patient group. Transfusion transmission of potential parasitic pathogens continues to be problematic. Transfusion in general is becoming more widely recognized as a source of infection, and donors are also more likely to come from many parasite-endemic areas of the world. It is also important to recognize the many neglected parasitic infections seen within the United States; indeed, the world continues to shrink in terms of infectious diseases.

With expanding regulatory requirements related to the disposal of chemicals, laboratories are continuing to review the use of mercury compounds as specimen fixatives and learning to become familiar with organism morphology when using substitute compounds. Permanent staining of fecal smears confirms
that none of the substitute fixatives provide results of the same quality found with the use of mercuric chloride-based fixatives. However, the key issue is whether the intestinal parasites can be identified using these alternative fixatives, not how “perfect” they look. Many fixative options are now available, including single-vial collection systems, some of which are coupled with their own stains. Requirements also mandate that any laboratory using formalin must have formalin vapor monitored as both an 8-hour time-weighted average and 15-minute readings. Most laboratories are now familiar with the regulations on protection of health care workers from blood and other body fluids and have implemented specific changes that are no longer optional. Although laboratories were already using many of the safety recommendations, these regulations delineate in detail what must be done and documented. Regulatory information based on new shipping requirements is also included.

On the basis of excellent suggestions and comments, I have made the following changes in this new edition: (i) the chapter on case histories has been expanded and contains a large number of parasite medical case histories (case history, study questions, correct answer and discussion, and illustrative material); (ii) some of the life cycles have been redrawn, and new life cycles have been added; (iii) algorithms have been expanded; (iv) new tables and figures have been added throughout the book; (v) additional drawings and photographs have been added; (vi) extensive color images have replaced the black and white images; (vii) extensive updated text information is included, all of which was taken from a comprehensive literature review of all aspects of diagnostic medical parasitology; (viii) additional examples of unusual parasitic infections are included; (ix) the chapter on arthropods has been expanded and includes additional photographs and drawings and expanded text; (x) the chapter on the immunology of parasitic infections has been enlarged, and updated information on both antigen and antibody detection methods continues to be included in this edition; (xi) the chapter on histological identification of parasites has been dramatically expanded with diagrams of various parasites and their visual presentations in tissue sections, with greatly enhanced legends for all images; (xii) diagnostic methods using newer immunoassay and “dipstick” technology are included; and (xiii) the chapter on quality control has been expanded to include information on instrumentation and equipment, safety regulations, quality control and quality systems information, continuous quality improvement, and managed-care considerations. The appendixes have been expanded to contain more information on artifacts; expanded lists and photographs of products and commercial suppliers; algorithms for ordering specific tests that complement the ova and parasite examination; flowcharts for processing stool specimens; quality control recording sheets for use in the laboratory; and general references and relevant web sites. One of the most important expanded areas of the sixth edition is found in Appendix 7, which contains information that has been published within months prior to the final printing of this edition. This “late-breaking” synopsis of very recent publications can assist the reader in having access to the latest information available. I encourage you to review this section as you read various chapters throughout the book. A more comprehensive discussion of molecular methods has also been added to the sixth edition and can be found in Appendix 8. Appendix 9 contains comprehensive information on the most frequently asked questions for all aspects of human parasitology, and Appendix 10 contains information related to CPT coding for testing options for diagnostic parasitology.

The approach to the sixth edition of the book has been revised to present the diagnostic methods first, then the didactic discussion of parasitic infections
as the second component of the book. This change was made to ensure that the most recent and relevant material would be updated right before editing. My objective is to provide the user with clear, concise, well-organized, clinically relevant, cost-effective, and practical quality procedures for use in the clinical laboratory setting. To use and fully understand these methods for the parasites discussed, it is imperative that the user also understand information related to life cycle, morphology, clinical disease, pathogenesis, diagnosis, treatment, epidemiology, and prevention. My intent is to provide a comprehensive discussion of both aspects of the field of diagnostic medical parasitology: first, relevant diagnostic methods designed to detect and identify the organisms present, and second, a comprehensive discussion of the individual parasites. I believe that the book fulfills these objectives and provides readers, whether they are laboratorians, physicians, or other health care professionals, with not only comprehensive, but very practical information.

It is also important for readers to understand that there are many diagnostic test options available to the clinical laboratory; not every laboratory will approach the diagnosis of parasitic infections in the same way. The key to quality and clinically relevant diagnostic work is a thorough understanding of the pros and cons of each option and how various options may or may not be relevant for one’s particular geographic area, laboratory size and range of expertise, client base, number and type of patients seen, personnel expertise and availability, equipment availability, educational initiatives, and communication options, just to name a few variables. However, it is also important to understand the regulations and technical recommendations that govern and guide this type of laboratory work; many of these guidelines are related to coding and reimbursement, proficiency testing, and overall clinical relevance.

The use of product names is not intended to endorse specific products or to exclude substitute products. Also, because of possible advances and changes in the therapy of parasitic infections, independent verification of drugs and drug dosages is always recommended. The diagnostic procedures are intended for laboratory use only by qualified and experienced individuals or by the personnel under their direct supervision. Every effort has been made to ensure accuracy; however, ASM Press and I encourage you to submit to us any suggestions, comments, and information on errors found.
Acknowledgments

Peter Schantz, Frederick L. Schuster, James Seidel, Nicholas Serafy, J. A. Shadduck, Harsha Sheorey, Irwin Sherman, Robyn Shimizu, Balbir Singh, James Smith, Rosemary Soave, Frank J. Sorvillo, S. L. Stanley, Jr., John Steele, Deborah Stenzel, Damian Stark, Linda Sterzenbach, Charles Sterling, James J. Sullivan, Alex Sulzer, Kevin S. W. Tan, Egbert Tannich, Herbert Tanowitz, Mehmet Tanyuksel, William Trager, Peter Traynor, Antonio R. L. Teixeira, Sam Telford, William Trager, Allan R. Truant, Jerrold Turner, Saul Tzipori, Jacqueline A. Upcroft, Peter Upcroft, Tom van Gool, Eric Vanderslice, Jacob Verweij, Govinda Visvesvara, Marietta Voge, Susanne Wahlquist, Kenneth Walls, Rainer Weber, Wilfred Weinstein, Louis Weiss, P. P. Wilkins, John Williams, John Wilson, Marianna Wilson, Jeffrey J. Windsor, Washington Winn, Martin Wolfe, Donna Wolk, Johnson Wong, Lihua Xiao, Nigel Yeates, Judy Yost, Wenbao Zhang, Charles and Wiladene Zierdt, and many others whom I may have failed to mention specifically. If the information contained in this edition provides help to those in the field of microbiology, I will have succeeded in passing on this composite knowledge to the next generation of students and teachers.

Special thanks go to Sharon Belkin for her additional illustrations for this edition. I also thank Ronald Neafie from the Armed Forces Institute of Pathology for providing many photographs to illustrate several areas of the book, particularly the information on histological identification of parasites, and Herman Zaiman for providing slides that he has prepared and/or edited from many contributors worldwide. Very special thanks go to the group at the Centers for Disease Control and Prevention for the use of many of their clinical parasitology images; these images are invaluable to the microbiology community and include images contributed to CDC by many others, as well.

I would like to thank members of the editorial staff of ASM Press, especially Ellie Tupper; they are outstanding professionals and made my job not only challenging but fun.

Above all, my very special thanks go to my late husband, John, for his love and support for the many projects that I have been involved in over the years. I could never have undertaken these challenges without his help and understanding, a true partnership.