

**A LABORATORY MANUAL
FOR THE
ISOLATION AND
IDENTIFICATION
OF AVIAN
PATHOGENS**



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**Fourth
Edition**

Editors

David E. Swayne, Chairman
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A Laboratory Manual for the Isolation and Identification of
Avian Pathogens

TABLE OF CONTENTS

Contributing Authors

Preface

1. Principles of Laboratory Diagnosis. Frederic J. Hoerr 1

Bacterial Diseases and Pathogens

2. Salmonellosis. W. Douglas Waltman, Richard K. Gast, and Edward T. Mallinson 4
3. Colibacillosis. Margie D. Lee and Lawrence H. Arp 14
4. Pasteurellosis, Infectious Serositis, and Pseudotuberculosis. Richard B. Rimler, Tirath S. Sandhu, and John R. Glisson 17
5. Bordetellosis. J. Kirk Skeeles and Mark W. Jackwood 26
6. Infectious Coryza. Pat J. Blackall and Richard Yamamoto 29
7. Campylobacteriosis. Simon M. Shane and Kathleen S. Harrington 35
8. Spirochetosis. H. John Barnes and David E. Swayne 40
9. Erysipelas. George L. Cooper and Arthur A. Bickford 47
10. Listeriosis. George L. Cooper and Arthur A. Bickford 51
11. Staphylococcosis. Marcus M. Jensen and J. Kirk Skeeles 55
12. Streptococcosis. Dennis P. Wages 58
13. Clostridial Diseases. David A. Miller 61
14. Tuberculosis. Charles O. Thoen 69
15. Mycoplasmosis. Stanley H. Kleven 74
16. Chlamydiosis. Arthur A. Andersen 81
17. Ornithobacteriosis. Richard P. Chin and Bruce R. Charlton 89

Fungal Diseases and Pathogens

18. Mycoses and Mycotoxicoses. Robert A. Kunkle and John L. Richard 92

Viral Diseases and Pathogens

19. Adenoviruses. J. Brian McFerran 100
20. Hemorrhagic Enteritis of Turkeys and Marble Spleen Disease of Pheasants. Frank W. Pierson, Charles H. Domermuth and W. Burnham Gross 106
21. Infectious Laryngotracheitis. Deoki N. Tripathy 111
22. Marek's Disease. Jagdev M. Sharma 116
23. Duck Virus Enteritis. Peter R. Woolcock 125
24. Herpesviruses of Free-Living and Pet Birds. Erhard F. Kaleta 129
25. Pox. Deoki N. Tripathy and Willie M. Reed 137
26. Budgerigar Fledgling Disease and Other Avian Polyomavirus Infections. Phil D. Lukert and Branson W. Ritchie 141
27. Psittacine Beak and Feather Disease. Phil D. Lukert and Branson W. Ritchie 144
28. Chicken Anemia Virus. M. Stewart McNulty 146
29. Influenza. David E. Swayne, Dennis A. Senne and Charles W. Beard 150
30. Newcastle Disease Virus and Other Avian Paramyxoviruses. Dennis J. Alexander 156
31. Avian Rhinotracheitis (Pneumovirus). Richard E. Gough, Dennis J. Alexander and Peter J. Wyeth 164
32. Infectious Bronchitis. Jack Gelb, Jr. and Mark W. Jackwood 169
33. Enteric Viruses. Don L. Reynolds 175
34. Oncornaviruses: Leukosis/Sarcoma and Reticuloendotheliosis. Aly M. Fadly and Richard L. Witter 185

35. Encephalomyelitis. Louis van der Heide	197
36. Duck Hepatitis. Peter R. Woolcock	200
37. Turkey Virus Hepatitis. Willie M. Reed	205
38. Viral Arthritis/Tenosynovitis and Other Reovirus Infections. John K. Rosenberger, Norman O. Olsen, and Louis van der Heide	207
39. Arbovirus Infections. James E. Pearson	211
40. Infectious Bursal Disease. John K. Rosenberger, Y.M. Saif, and Daral J. Jackwood	215
41. Goose Parvovirus (Derzsy's Disease). Richard E. Gough	219

Standard Laboratory Procedures

42. Cell-Culture Methods. Karel A. Schat and H. Graham Purchase	223
43. Virus Propagation in Embryonating Eggs. Dennis A. Senne	235
44. Virus Identification and Classification. Pedro Villegas and Phil D. Lukert	241
45. Titration of Biological Suspensions. Pedro Villegas	248
46. Serologic Procedures. Stephan G. Thayer and Charles W. Beard	255
47. Molecular Identification Procedures. Daral J. Jackwood and Mark W. Jackwood	267
48. Antigen Detection Systems. Sandra S. Cloud	270

Appendix of Abbreviations and Acronyms used in the Text	278
--	-----

Appendix of Reference Antisera and Other Reagents	281
--	-----

Quick Reference Diagnostic Chart	289
---	-----

Appendix of Sources	302
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Index	306
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PREFACE

This manual has its origins in the need for a book to codify standardized method for testing and evaluating poultry vaccines. The National Academy of Sciences sponsored the original publication entitled *Methods for the Examination of Poultry Biologics*, and completed two successive revisions. In 1975, the American Association of Avian Pathologists (AAAP) accepted responsibility for the publication, but because the need for standardizing testing of vaccines had been met, the scope and purpose of the manual was broadened to be a resource for laboratory procedures for the isolation and identification of disease-causing agents. The title was changed to *Isolation and Identification of Avian Pathogens* for the 1st edition and to *A Laboratory Manual for the Isolation and Identification of Avian Pathogens* for the 3rd edition. The manual was intended as a bench resource for daily use in the diagnostic laboratory.

With the 4th edition, the focus has shifted from a manual of procedures for isolation and identification of pathogens to a more encompassing manual for diagnosis of the disease and isolation and/or demonstration of the pathogen. This change was needed because many clinical specimens are presented as unknowns for determination of etiology and some pathogens are difficult to isolate, but can be demonstrated with newer molecular or immunologic techniques. With the 4th edition, the AAAP appointed four new editorial committee members to replace Lawrence H. Arp, Charles H. Domermuth, and H. Graham Purchase. John R. Glisson, Willie M. Reed, and Mark W. Jackwood were appointed to provide expertise in bacteriology, veterinary diagnostics, and molecular biology, respectively. David E. Swayne was appointed chairman of the editorial committee to succeed H. Graham Purchase, chairman for the 3rd edition. James E. Pearson was reappointed for the 4th edition to provide continuity and expertise in virology.

The 4th edition has several significant changes including five new chapters: Principles of Laboratory Diagnosis, Ornithobacteriosis, Goose Parvovirus (Derzsy's Disease), Molecular Identification Procedures, and Antigen Detection Systems. Six chapters were reorganized and combined because of common themes or related agents: Reticuloendotheliosis and Leukosis Sarcoma were combined into a single chapter on Oncornaviruses, Turkey Meningo-Encephalitis and Arbovirus Infections were combined into a single chapter on Arbovirus Infection, and Enzyme Immunoassay for Poultry Disease Monitoring and Serologic Procedures were combined into a single chapter on Serologic Procedures. The Introduction, Logarithms, and McFarland Nephelometer chapters in the 3rd edition were deleted. Improvements to individual chapters include the addition of a Summary section at the beginning of each chapter, and clarifying and redistributing information reported in the Serologic Identification section of the 3rd edition into two sections for the 4th edition: Agent Identification (serotyping or classifying of isolates) and Serologic Detection in the Host (detection of infection in the host).

Other improvements in the 4th edition include three additions to the appendix section: a quick reference chart on individual disease diagnoses, a list of acronyms used in the text, and a resource list of reference antisera and other reagents as compiled by the Reference Antisera Committee, AAAP. The Appendix of Sources now includes, where available, fax and telephone numbers, and email and internet addresses of companies supplying specialized equipment and reagents. Furthermore, the author list includes fax numbers and email addresses for editorial committee members and senior authors.

The editorial committee thanks all of the 61 contributors who prepared new chapters or revised existing chapters for the 4th edition. Karen Hellekson, Kathy Early, and other staff at Allen Press, Lawrence, Kansas, are acknowledged for the copy-editing services. Debra Duckworth, Southeast Poultry Laboratory, is thanked for clerical support. Sue Clanton, University of Georgia, is gratefully acknowledged and thanked for format and editorial corrections and assembly of the book.

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PRINCIPLES OF LABORATORY DIAGNOSIS

Frederic J. Hoerr

SUMMARY

Poultry production has achieved worldwide importance and accurate and timely diagnosis of avian diseases is integral to poultry health, the global economy, and consumer safety. Multiple disease agents and management factors may be identified in laboratory investigations; establishing their relative importance is important in the development of effective control strategies. Molecular biology continues to offer new diagnostic tools, and many infectious avian pathogens are now routinely identified by these procedures. Quality assurance and control programs and standard operating procedures improve the overall accuracy of laboratory diagnosis. Implicit with isolation and identification of certain pathogens is the responsibility of reporting certain diseases to regulatory officials. Many avian pathogens and the reagents used in their identification pose health risks to laboratory workers, and appropriate training and safeguards are required. Proper archiving of avian pathogens acquired from diagnostic investigations is important for studies in comparative pathology and epidemiology, and some pathogens may be candidates for vaccine development.

INTRODUCTION

During the past century, the emerging dominance of poultry in food animal production worldwide has borne witness to the emergence of the diagnosis of avian diseases from a comparative medical perspective to becoming a necessity for the well-being of poultry health, the global agricultural economy, and consumer safety. Given the large scale of agribusiness production, the vulnerability of large populations of at-risk animals, and the shifting social perspective of food safety, the accuracy and timeliness of diagnostic information has become pivotal.

The usual immediate challenge is to find a definitive primary diagnosis for the presenting problem of morbidity or mortality. For many cases, the deeper the investigation proceeds, the more likely it is to yield evidence of concurrent or predisposing infectious diseases, management or nutritional problems, and a variety of other conditions that underlie the presenting problem. From the outset, the avian diagnostic process requires sound methods because the results will be evaluated not just for pathogenic implications but also for economic impact on an agribusiness system. Conversely, it will become quickly evident if the focus of control is based on a secondary diagnosis or a misdiagnosis. This merger of science and economics is a challenging and exciting feature of avian medicine in commercial poultry production. This text deals exclusively with diagnostic procedures, but the reader should be aware that laboratory results will undergo economic considerations in the development of strategies for treatment, prevention, and control.

Three factors that influence the expression of any infectious disease are the virulence of the causative organism, the level of exposure or dose of the inoculum, and

the susceptibility of the host. Within a population such as a poultry flock, each individual will react according to the net effect of these factors. In commercial food animal agriculture, the uniformity of these factors among individuals in the flock will eventually influence performance parameters and possibly food safety. Commercial poultry experience many sequential and sometimes simultaneous disease challenges, as well as concurrently applied efforts to assist them in resisting those challenges, such as attenuated live vaccines, antibiotics, and antiparasitic drugs. For meat-type chickens, ducks, and turkeys, all of this occurs essentially to neonatal animals that require one third to one half of their short life span to acquire functional maturity of the immune and digestive systems and never acquire a mature skeletal system.

Fortunately, much is known about isolation and identification of infectious and noninfectious disease agents, and the nature of their interactions. In an appropriately equipped laboratory, it is possible to establish a scheme of laboratory analysis to confirm or rule out involvement of the various agents in common clinical diseases. Still, there are limits to what can be learned by laboratory analysis. Birds may have lesions suggestive of mismanagement and laboratory testing cannot replace on-site evaluation of management practices to define the actual problem and develop the appropriate corrective strategy.

HISTORICAL PERSPECTIVE

The title of this book, *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*, emphasizes the importance of infectious diseases that affect poultry and other birds. Not so long ago, disease was classified mostly by clinical signs and postmortem pathology. Agents visible

INDEX

Numbers in bold represent major section, page numbers with *t* indicates table and page numbers followed by *d* are differential diagnoses.

- Acholeplasma, 75, 76
 Adeno-associated, 102, 104d, 242
 Adenovirus, 42d, 62d, 66d, **100**, **106**, 133d, 154d, 206d, 210d, 242t, 245, 272
 Aflatoxicosis (see Mycotoxicosis), 204d, 297T
 Airsacculitis, 14, 22, 74, 89
 Anaerobic bacteria, 61
 Anatid herpes, 125
 Anatipestifer syndrome, **22**
 Anemia agent (see Chicken anemia virus)
 Anemia dermatitis syndrome, **146**
 Antigen capture, 108, 152, 271
 Arbovirus, **211**, 242t
 Arbovirus infection, **211**
 Arizonosis, 4, 73d
 Aspergillosis, 12d, 53d, 73d, 92
 Aspergillus, 92
 Avian encephalomyelitis, 53d, 63d, 95d, **197**, 214d, 235d, 242t, 258, 271
 Avian influenza (AI), 2, 49d, 79d, 126d, 133d, **150**, 167d, 173d, 204d, 243, 249d, 258
 Avian leukosis, **185**, 232, 237
 Avian pneumovirus, 2, 156, **164**
 Avian rhinotracheitis, **164**, 242t
 Avian pox, 137
 Astrovirus, **175**, 200, 242t
 Avipoxvirus, **137**, 242t
 Bacillus, 53, **61**, 133
 Barnhardt variant DHV type I, **200**
 Beak-and-feather disease, 142d, **144**, 242t
 Big liver disease, **185**
 Birnavirus, **215**
 Bluecomb, **176**, 285
Bordetella avium, **26**, 264
 Bordetellosis, 21d, **26**
 Borrelia, **40**
 Botulism, **62**, 133d
 Brooder pneumonia, 92
 Bronchitis (see Infectious bronchitis)
 Budgerigar fledgling, **141**, 145d
 Bursal disease (see Infectious bursal disease)
 Bursitis, 12d
 Calicivirus, 209, 242d
Campylobacter coli, **35**
Campylobacter jejuni, 2, **35**
Campylobacter lari, **35**
 Campylobacteriosis, **35**, 45, 133
 Candidiasis, 92, **94**
 Candida, 92, **94**
 Canarypox, c25
 Cell-associated virus, **118**
 Cellulitis, 14, 66
 Chicken anemia virus, **146**, 215, 217d, 242t, 264, 272
Chlamydia psittaci, **81**, 133d, 204d
 Chlamydiosis, 16d, 23d, 28d, 42d, 73d, 79d, **81**
 Cholera, 17, **18**, 33d, 49d, 57d, 63d, 74d, 79d, **81**
 Chronic respiratory disease, 33d
 Ciliostatic agent, **164**
 Circovirus, 241, 242t
 Citrobacter, 10, 11
 Clostridial infections, **61**
 Clostridium, 45d, **61**
Cl. botulinum, **62**, 64
Cl. colinum, **63**, 66
Cl. difficile, 64
Cl. novyi, 66
Cl. perfringens, 64, **65**
Cl. septicum, **66**
Cl. sordelli, **66**
Cl. sporogenes, **66**
Cl. subterminale, **66**
 Coccidiosis, 2, 45d, 63, 64d, 66d, 108d
 Cockatoo, 144
 Colibacillosis (see also Escherichia)
 Colisepticemia, 14, 53d
 Coronaviral enteritis, 175, **176**
 Coronavirus, **169**, **176**, 242t, 243
 Coryza (see Infectious coryza and Turkey coryza),
 Crop mycosis, **94**
 Cryptosporidiosis, 28d, 79d
 Dactylaria, **95**
 Dactylariosis, **95**
 Delayed secondary enrichment, 4, 9
 Dermatocycosis, **92**
 Dermatophyte, **92**
 Derzsy's, **219**
 Disease reporting, 1
 Drag swabs, 7
 Duck hepatitis, **200**, 221d
 Duck hepatitis B virus, **200**, 221d
 Duck plague, 19d, **125**, 242t
 Duck septicemia, **22**
 Duck virus enteritis, **125**, 204d
 Duck hepatitis, **200**, 221d
 Eendenpest, 125
 Egg-drop syndrome-76 (EDS-76), **100**, 242t, 243
 Encephalitis (Western and Eastern), 45d, **211**, 242t
 Encephalomalacia, 53d, 96d, 199d
 Encephalomyelitis (AE), 53d, 63d, 95d, **197**, 235, 242t, 258, 271

- Encephalomyelitis virus, **197**
 Entenpest, 125
 Enteric viruses, **175**
Enterobacter, 15
Enterococcus avium, **58**
Enterococcus durans, **58**
Enterococcus faecalis, **58**
Enterococcus faecium, **58**
Enterococcus hirae, **58**
 Enterovirus, **175**, 198, **201**
 Environmental sampling, 7
 Epidemic tremor, 197
 Erysipelas, 16d, **47**, 53d, 60d, 108d, 209d
 Erysipeloid, 47
Erysipelothrix rhusipathiae, 3, 47, 53d, 133d
 Escherichia coli (see also Colibacillosis), 2, 11, 12d, **14**, 23d, 26, 38d, 56d, 66, 79d, 97, 133d, 164, 173d, 206d
 Esophagitis, 131t
 Favus, **96**
 Flavivirus, **211**, 242t
 Food safety, **1**
 Free-living birds, 35, **129**
 French molt, 141, 142, 242t
 Fowl pest, **150**
 Fowl plague, **150**
 Fowl pox, 33d, 114d, 122d, **137**, 186, 242t, 258
 Fusarium, 97
 Gangrenous cellulitis, 66
 Gangrenous dermatitis, **66**, 217d
 Gangrenous dermatomyositis, 66
 Geese, 21, 40, 65, 81, 100, 125, 131t, 157t, **219**
 Gliotoxin, 96, **98**
 Goose hepatitis, **219**, 242t
 Goose hepatitis virus, **219**
 Goose parvovirus, **219**, 242t
 Gosling plague, 219
 Gumboro, **215**
 H antigens, 38
Haemophilus paragallinarium, **29**
 Hemorrhagic aplastic anemia, 217d
 Hemorrhagic enteritis, 64, **100**, **106**, 212, 242t, 264
 Hemorrhagic syndrome, **146**
 Hepadnavirus, 200
 Hepatitis, 35, 81, 100, 127d, 131t, 147, **201**, 218d, 222, 243t
 Hepatosplenitis, 131t
 Herpesvirus, 113, **116**, **125**, 129, 140d, 186, 221, 242t
 Herpesvirus of turkeys, 117
 Highlands J virus, 211
 Hyaluronidase, 33, 49
 Hydropericardium syndrome, 100
 Hypovitaminosa A, 33d
 IBDV, **215**, 217d, 271
 Immunosuppression, 97, 106, 116, 148d, 185, 215, 217d
 Inclusion bodies, 111, 126, 142d, 205, 210d, 233
 Bollinger and Borrell, 138
 Cowdry, 132
 Intranuclear, 42d, 102, 140d
 Inclusion body hepatitis, 100, 131t, 217d, 242t
 Infectious anemia, 146
 Infectious bronchitis (IB), 79d, 93d, 114d, **169**, 242t
 Infectious bronchitis virus, 26, 105d, 164, 167d, **169**, 179, 217d, 237, 256, 271
 Infectious bursal disease (IBD), 92, 173d, 194, **215**, 242t
 Infectious bursal disease virus, 66, **216**, 237, 271
 Infectious coryza, 2, 19d, **29**, 93d, 173d, 263
 Infectious laryngotracheitis (ILTV), 1, **111**, 129, 131t, 137, 140d, 242t
 Infectious laryngotracheitis virus (ILTV), **111**, 122d, 173d, 210, 264, 272
 Influenza (AI), 2, 49d, 79d, 126d, 133d, **150**, 167d, 173d, 204d, 243, 249d, 258
 Influenza virus, 127d, **150**, 159, 162d, 167, 204d, 239, 256, 272
 Intoxication, 62, 63d, 96, 217d
 Isolation and identification, **1**, 19d, 21d, 23d, 25d, 38d, 53d, 67d, 70, 79, 82, 87d, 91d, 173d, 176, 209, 214d, 221
 Klebsiella, 15, 133
 Laboratory safety, **3**
 Lake Victoria cormorant virus, 130, 131t
 Laryngotracheitis (see Infectious laryngotracheitis)
 Laryngotracheitis virus (see Infectious laryngotracheitis virus)
 Latent infection, 112, 119, 125
 Lentogenic, 159, 160t, 162
 Leukosis, 42d, 107, 122d, **185**, 188t, 193d, 237, 242t, 264
 Leukosis/sarcoma virus, **185**, 188t, 189, 232, 237
 Limberneck, 62
 Listeria, 49d, **51**, 53t
Listeria monocytogenes, 49d, **51**, 53t, 59
 Listeriosis, **51**
 Litter sampling, 7, 36
 Logarithms, 2, 251
 Lymphoid leukosis, 107, 122d, **185**, 264
 Lymphoma, 185, 186
 Lymphoproliferative disease, 108d, 191
 Malabsorption syndrome, 176, **207**
 Malignant edema, 66
 Management, 1, 26, 105, 194, 263
 Marble spleen, **100**, **107**
 Marek's disease, 42d, 53d, 63d, 107, **116**, 130, 186, 209, 217d, 242t, 243, 258
 Marek's disease virus, **116**, 129, 147, 232
 McFarland nephelometer, 10

- Media, solutions and reagents (see also Transport media)
- Agar overlay, 190, 227, 229t, 233
- Andrade's, 49
- Antibiotic solution, 84, 225
- API 20 C yeast identification, 95
- Bacitracin, 30, 36
- Bacto skim milk, 8
- BG sulfa, 10
- Bile esculin, 59
- Bismuth sulfite, 8, 10
- Blaser's, 36
- Bovarnick's, 84
- BME, 130, 203, 228t
- Brain-heart infusion (BHI), 9, 15, 27, 31, 44, 48, 51, 151, 237
- Brilliant green (BG), 9, 10
- Broth enrichment for *Campylobacter* spp. (BEM), 36
- Butzler's, 36
- Cephalothin, 36
- Chicken embryo extract, 186, 227
- Chlamyospore, 94
- Chloroform, 105d, 117, 132, 146, 147, 171, 181, 198, 202, 204d, 206, 208, 212, 216, 217, 220, 243, 247t
- Chocolate, 23
- Colistin, 36, 43
- Columbia CNA, 56
- Cornmeal, 94
- Cycloheximid, 36, 84, 85, 93
- Czapek's solution, 93
- Decontaminant for Mycobacteria, 70
- Dextrose starch, 18
- Dulbecco's balanced salt, 224, 225t
- Eagle's basal, 224, 229t
- Earle's balanced salt, 224, 229t
- Erysipelothrix selective, 47, 48
- Frey's, 77t
- F10-199 culture, 228t, 229t, 230
- Gentamicin, 23, 82, 84, 90, 126, 151, 186, 203, 226, 231, 237
- Glycerol, 14, 20t, 22, 70, 86, 102, 181, 198
- Hajna, 9
- Hajna and Damon, 9
- Hank's balanced salt, 112, 138, 142, 203, 224, 225t, 226, 228t, 231
- Hektoen enteric, 8, 10
- Haemophilus maintenance, 30
- Ink dye, 93
- Kligler's iron, 15
- Lactophenol cotton blue mount, 93, 94
- Lactalbumin hydrolysate, 209, 213, 224, 228t, 229t
- Liebovits-McCoy (LMH), 112, 230
- Lior's, 38

- Media, solutions and reagents (cont.)
- Lowenstein-Jensen, 71
- Lysine iron (LI), 10, 11
- MacConkey, 9, 20t, 26, 27, 90t
- Mannitol-salt, 11t, 20t, 32t, 49, 56
- Mayer's albumin fixative, 93
- Middlebrook 7H9, 70
- Monoclonal antibodies, 53, 85, 86, 114, 118, 133, 148, 159, 161, 167d, 171, 187, 191, 194, 241, 273,
- Mucopolysaccharides, 19
- Mueller-Kauffmann, 9
- Mycobiotic, 94, 96
- Mycosel, 94, 96
- M20, 228t, 229t, 232
- Neutral red solution, 225, 227, 229t
- Niacin test, 71t
- Nitrate reduction, 72
- Novobiocin supplemented, 8
- Packer's, 48
- Paterson and Cook, 24t
- Peptone, 8, 30, 35, 151, 209
- Phenylethyl alcohol, 56
- Phosphate-buffered saline, 27, 44, 45, 78, 84, 85, 102, 117, 152, 164, 176, 186, 198, 220, 224, 225t, 256
- PPLO broth, 77t
- Potato dextrose, 93
- Proskauer-Beck, 70
- Sabouraud's, 93
- Selenite, 9, 37, 231
- SIM, 15
- Skirrow's, 36
- SPG buffer, 82
- Staphylococcus, 56
- Sucrose-phosphate-glutamate-albumin (SPGA-EDTA), 117, 130
- Sucrose-phosphate-glutamate (SPG), 82, 84
- Supplemented test (TM/SN), 30, 31
- Tellurite glycine, 56
- Tergitol, 8, 9, 15
- Tetrathionate, 8., 9
- Trimethoprim, 36
- Triple sugar iron (TSI), 10, 11, 15, 48, 49, 52, 90t
- Trypticase soy, 18, 23, 24, 51, 59
- Tryptose, 15, 18, 19, 23, 48, 59, 151, 237, 248
- Tryptose phosphate, 64, 120, 142, 170, 190, 203, 215, 226, 228t, 229t
- Trypsin, 90t, 138, 153, 158, 160, 177, 202, 224, 273
- TT, 8, 9
- Tween 80 hydrolysis, 71, 72t
- Xylose-lysine-desoxycholate (XLD), 8, 10
- Meningoencephalitis, 211, 242t
- Mesogenic, 159, 160t
- Micrococcus, 56
- Microsporium, 96

- Molecular biology, 1, 224, 268
- Molecular probes, 95
- Muscovy ducklings, 126, 219
- Muscovy duck parvovirus, 221
- Mycobacterium, 69, 133
- Mycobacterium avium*, 69, 71
- Mycoplasma, 56d, 74, 75t, 77t, 79d, 87d, 93d, 105d, 132, 154d, 164, 167, 169, 173d, 182, 209d, 226, 237, 256, 260, 263, 267
- Mycoplasmosis, 16d, 21d, 28d, 74, 91d, 114d, 173d, 256, 258, 263
- Mycosis, 92, 94
- Mycotoxin, 92, 95d, 209
- Mycotoxicosis, 96, 173d
- Mynah pox, 139d
- Necrotic enteritis, 64d, 65, 66d
- Necrotic dermatitis, 66
- Neoplasms (see also Leukosis sarcoma, Marek's disease, Reticuloendotheliosis), 73d, 190
- Neuraminidase, 49, 151, 153, 158, 165, 167, 171
- Neurotoxin, 61
- Newcastle disease, 12d, 26, 28, 49d, 53d, 63d, 79d, 156, 167, 173d, 199
- Newcastle disease virus (see also Paramyxovirus), 105, 122, 127d, 133d, 152, 154d, 156, 157t, 169, 204d, 214d, 237, 242t, 243, 256, 272
- New duck disease, 22
- Notification of State & Federal Agencies, 22, 156
- Nurse cultures - Haemophilus, 30
- Nutrition, Deficiencies, 33d, 56d, 67d, 183d
- O antigens, 38
- Ochratoxin, 96
- Oncornaviruses, 122, 185, 193d
- Ornithobacterium rhinotrachealae*, 32, 89, 90t, 91d
- Ornithobacteriosis, 89
- Ornithosis (see Chlamydiosis), 81
- Orthomyxovirus, 133d, 150, 239
- Osteopetrosis, 185
- Osteoporosis, 207, 208
- Pacheco's disease, 129, 131t, 242t
- Pancreatitis, 199, 208
- Papovavirus, 133d, 141
- Paralysis (flaccid), 24, 41, 62, 141, 197, 199, 212
- Paramyxovirus, 129, 133, 152, 154d, 156, 157t, 162d, 165, 167, 199d, 239, 242t
- Paratyphoid, 4, 11t, 73d
- Parrot fever, 81
- Parvovirus, 102, 133d, 144
- Pasteurella hemolytica*, 21, 66, 89
- Pasteurella multocida*, 18, 19d, 20t, 53d, 56d, 127d, 206d, 222d, 264
- Pasteurellosis, 2, 16d, 42d, 49d, 53d, 57d, 60d, 108d, 127d
- Penicillium, 97
- Pet birds, 129, 242t
- Pheasants, 22, 41, 47, 62, 63, 100, 106, 112, 129, 131t, 191, 194, 197, 205, 211
- Phospholipase C, 171
- Picornavirus, 200
- Picornavirus-like, 205
- Pigeon pox, 137, 139d, 242t
- Plague, 19d, 125, 150, 219, 242t
- Pneumovirus, 2, 28d, 156, 164, 242t
- Polyomavirus, 141, 142d, 145d, 242t
- Poxvirus, 127d, 137
- Poult enteritis, 176
- Poult enteritis and mortality syndrome (PEMS), 176
- Pox, 33d, 95d, 137, 139d, 140d, 242t
- Procedures
- α procedure, 258
- Actinomycin D sensitivity, 245t, 247t
- Agar gel immunodiffusion test (AGID), 44, 91, 139, 162
- Agar gel precipitin (AGP), 107, 216
- Agglutination, 11, 12, 19, 23, 24, 28d, 31, 42, 49, 60, 75, 7i8, 79, 91, 95, 161, 162d, 179, 182, 256, 263, 271
- Allantoic sac inoculation, 151, 237, 238
- Amniotic sac inoculation, 237, 239
- Antiserum, 11, 23, 49, 76, 78, 85, 102, 103, 105, 108, 126, 133, 139, 147, 152, 154d, 158, 162, 165, 171, 180, 190, 216, 221, 258, 271
- Antigen capture, 152, 271
- Antigen capture ELISA, 108, 271
- Antigen preparation, 86, 179, 258, 272
- Anton test, 52, 53d
- API 20 C yeast identification, 95
- β procedure, 257
- CAM inoculation, 112, 118, 126, 132, 137, 178, 201, 208, 210d, 216, 235
- Catalase, 23, 27, 32t, 53, 56, 59, 90t
- Cell culture, 82, 102, 105, 107, 112, 118, 130, 133, 138, 142, 153, 158, 165, 170, 178, 187, 192, 198, 203, 206, 208, 212, 223, 228t, 241, 250, 260
- Cell lines, 84, 119, 130, 212, 216, 224, 231
- Chicken embryo fibroblast, 118, 166, 208, 217, 243
- Chicken embryo kidney, 104, 112, 118, 130, 138, 230, 274
- Chorioallantoic membrane inoculation, 235
- Coagulase test, 56
- Complement fixation, 35, 64, 85, 86, 133, 186, 190, 191, 233
- Cross-challenge test, 216
- Decontamination, 70t
- Delayed secondary enrichment, 9

- Procedures (cont.)
 Dilutions, 27, 28, 44, 76, 78, 79, 102, 114, 118, 127, 133, 142, 151, 159, 167, 171, 180, 182, 183, 193, 199, 201, 203, 209, 213, 217, 221, 233, 248, 254t, 256
 DNA inhibitors for virus nucleic acid determination, 245
 DNA hydrolysis test, 38
 Drag swabs, 7
 Egg inoculation, 177, 237
 Elementary body agglutination (EBA), 87
 Embryo (egg) inoculation, 112, 118, 132, 138, 205, 208, 216
 Enrichment, 5, 36, 51, 63, 226, 271
 Enzyme-linked immunosorbent assay (ELISA), 10, 19, 27, 45, 63, 72, 79, 85, 91, 93, 104, 108, 112, 116, 119, 121, 133, 139, 148, 152, 162, 166, 172, 179, 182, 186, 198, 221, 233, 246, 263, 268
 Electron microscopy, 37, 42, 102, 113, 126, 132, 145, 159, 165, 179, 202, 219, 235, 240, 246, 270
 Filtration, 8, 36, 84, 102, 117, 118t, 132, 151, 224, 246, 271
 Flock profiling, 44, 148
 Freezing cells, 82, 101, 130, 187, 232
 Geometric mean titers, 251, 254t, 265
 Guinea pig inoculation, 52
 Harvesting virus, 27, 78, 178, 231, 240
 Hemagglutination, 19, 27, 28d, 79, 101, 104, 127, 133, 139, 152, 158, 165, 170, 179, 213, 235, 247t, 258, 271
 Hemagglutination inhibition, 171, 182, 256
 Hippurate hydrolysis, 37t, 38
 Hybridization with nucleic acid probes, 267
 Immunocytochemistry, 146, 152, 194, 216
 Immunodiffusion, 10, 41, 91, 105, 108, 112, 139, 152, 162, 166, 183, 209, 221, 258, 271
 Immunoelectron microscopy, 102
 Immunofluorescence, 10, 42, 53, 76, 102, 120, 126, 133, 142, 147, 166, 188, 191, 203, 220, 233
 Immunohistochemical staining, 84, 101, 122, 224, 272
 Indole test, 19
 Infectivity assays, 248
 In situ hybridization histochemistry, 274
 Intracerebral pathogenicity index (ICPI), 159, 160t
 Intraocular pathogenicity test, 160
 Intravenous pathogenicity index (IVPI), 160t
 In ovo vaccination, 122
 Isolation procedure, 237, 258
 Kovac's indole test, 19
 Kovac's oxidase test, 19
 Lactophenol cotton blue mount, 93, 94
 Latex agglutination, 56, 60, 87, 179
 Lior serotyping of *Campylobacter*, 38
 Lipid solvent sensitivity, 188, 202

Procedures (cont.)

- Lymphocyte cultures, 230
 Lysostaphin sensitivity, 56
 McFarland nephelometer, 10
 Mean death time, 159, 160t
 Microaerobic culture, 36
 Microagglutination, 12, 27
 Microcomputer logarithms, 251
 Microcomputer for ELISA, 263
 Neutralization, 65, 104, 112, 114, 121, 133d, 139, 147, 153, 158, 165, 171, 181, 192, 198, 203, 209, 216, 221, 259, 263t
 Neutralization index, 198, 261, 263t
 Neuraminidase inhibition, 158
 Niacin production test, 71
 Nitrate reduction, 27, 72, 72t
 Non-producer cell activation (NP), 190
 Nuclease sensitivity, 243, 245t
 Nucleic acid detection, 102, 120, 139, 144, 153, 208, 213, 243, 245t, 267
 Nucleic acid sequencing, 141, 241, 269, 274
 Nucleic acid type, 102, 208, 243
 Oxidase test, 27
 Passage in eggs, 147, 152, 198, 212, 240
 Penner serotyping of *Campylobacter*, 38
 Phenotypic mixing (PM), 187, 189
 Polymerase Chain Reaction (PCR), 32, 78, 85, 108, 113, 114, 120, 141, 145, 148, 153, 165, 192, 216, 233, 267, 268
 Pullorum-typhoid test, 12
 Quantal assays, 248
 Rabbit inoculation, 23
 Random amplified polymorphic DNA (RAPD), 269
 Reed and Muench endpoint, 199, 249t, 262t, 263t
 Resistance-inducing factor (RIF), 187, 190, 193
 Restriction fragment length polymorphism (RFLP), 172, 267
 Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR), 153, 161, 172, 268
 Serological typing, 11
 Serum plate agglutination, 75, 78, 89
 Slide-smear, 113
 Spearman-Kärber endpoint, 250, 261
 Spot hemagglutination, 271
 Statistical analysis, 2
 Tellurite reduction, 72
 Thymidine analogs, 245t, 247t
 Titration of biologic suspensions, 161, 248
 Titration of HVT vaccine, 122
 Tracheal organ culture, 165
 Transformation (focus) assay, 190
 Trypsin activation, 177, 183
 Tween 80 hydrolysis, 71, 72t
 Urease, 11, 20t, 24, 27, 28t

Procedures (cont.)

- Virus isolation, 101, 112, 117, 125, 130, 141, 146, 151, 157, 164, 170, 176, 186, 193, 197, 199d, 212, 219, 233, 235, 271
 Virus neutralization (see Neutralization)
 Virus propagation, 205
 Virus titration, 138, 171, 178, 189, 193, 248, 260
 Whole blood test for *Salmonella*, 12, 263
 Yolk sac inoculation, 197, 238
 Proteus, 9, 11, 26
 Providencia, 9, 11
 Pseudomonas, 9
 Pseudomembranes, 65
 Psittacine, 81, 132, 141, 144, 157, 160
 Psittacine-beak-and-feather disease (PBFD), 144
 Psittacosis, 81
 Pseudotuberculosis, 16d, 19d, 20t, 21d, 24
 Pullorum disease, 4, 93d
 Quail bronchitis, 100
 Quail disease, 63
 Quail pox, 137
 Quality assurance, 1
 Quality control, 3, 265
 Reovirus, 105d, 171, 175, 181, 183, 206d, 207, 210d, 215, 222d, 237, 242t, 243, 264
 Reticuloendotheliosis, 168, 185, 242t
 Reticuloendotheliosis virus, 122, 185, 209, 232
 Retrovirus, 242t
 Rhinotracheitis, 28d, 156, 164, 214d, 242t
 Ringworm, 96
 Rotavirus, 176, 242t, 246
 Ruptured tendons, 56
 Salmonella, 4, 11t, 23d, 38d, 45d, 133, 183d, 206d, 209, 264
Salmonella pullorum, 4, 11t
Salmonella gallinarum, 4
 Salmonellosis, 4, 16d, 25d, 45d, 49d, 53d, 204d, 263
 Sarcoma, 10, 187, 190, 226, 242t
 Sarcomas (see Leukosis)
 Secondary agents, 67, 89, 164
 Selective enrichment, 5
 Sentinel chicken, 170
 Septicemia, 14, 16d, 22, 24, 29, 40, 55, 56d, 108d, 144
 Serology, 16, 42, 86, 101, 141, 172, 221, 264
 Serositis, 4, 22, 41, 42
 Smadel's disease, (not found)
 Spicer-Edwards flagella groups, (not found)
 Spirochetosis, 40
 Splenomegaly, 42, 58, 100, 107, 200
 Stains
 Acridine orange, 112, 138, 243, 245t
 Fluorochrome, 70
 Feulgen, 138
 Giemsa, 18, 44, 83, 85, 112, 113, 120, 138, 142
 Gimenez, 83, 85, 139

Stains (cont.)

- India ink, 18, 23, 93
 Ink dye, 93
 Kinyoun, 70, 71t
 Macchiavello's, 85
 May-Grunwald, 112
 Methylene blue, 71t, 93
 Pierce-Van Der Kamp, 83t, 85
 PVK, 83t
 Silver, 43, 181
 Wrights, 18, 44, 43
 Ziehl-Neelsen, 70
 Staphylococcosis, 16d, 49d, 53d, 55, 57d, 60d
 Staphylococcus, 30, 53, 55, 66, 209
S. aureus, 52, 55, 66, 79, 271
S. epidermidis, 55, 66
 Streptococcosis, 49d, 53d, 58
 Streptococcus, 53, 58
Streptococcus zooepidemicus, 58, 59t
 Stunting syndrome, 176, 207
 Stunting syndrome agent (SSA), 175
 Swans, 22, 125, 131t
 Swollen head syndrome, 156, 164, 167, 173
 Syndromes, 100, 185, 194, 207
 Syngamus, 133
 Synovitis, 4, 12, 19, 55, 74, 79d, 209
 Tenosynovitis, 58, 79, 208, 209
 Togavirus, 242t
 Transport media (see also Media)
 Anaerobic tubes, 64
 Antibiotic, 36t, 81, 84, 97, 130, 158, 178, 220, 224, 225, 228t, 229t, 237, 240, 245
 Brain-heart infusion, 9, 15, 27, 31, 44, 48, 51, 151, 237
 Copper loop, 151
 Dimethyl sulfoxide (DMSO), 117, 121, 130
 Dry filter paper, 226
 Eagle's basal medium (BME), 224, 229t
 Glycerine and broth, 93
 M199, 102, 130, 227, 228t, 229t, 231
 Nichrome loop, 151
 Nutrient, 9, 36t, 48, 51, 59, 70, 151, 183, 197, 205, 208, 237, 239, 248
 Paterson and Cook, 24t
 Phosphate-buffered saline, 27, 31, 36, 44, 78, 84, 102, 107, 117, 133, 147, 152, 158, 164, 176, 186, 198, 205, 212, 220, 224, 225t, 238, 244, 256, 271
 Port-A-Cul, 64
 Silica gel, 59
 Skim milk, 5, 8
 Stuart's, 36
 Sucrose-phosphate-glutamate (SPG), 82, 84
 Sucrose-phosphate-glutamate-albumin (SPGA), 117, 119, 130



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