

The Veterinary Laboratoryand Field Manual

3RD EDITION

Edited by Susan Cork, Roy Halliwell

world organisation for animal HEALTH Protecting animals, preserving our future

THE VETERINARY LABORATORY AND FIELD MANUAL

THE VETERINARY LABORATORY AND FIELD MANUAL

3RD EDITION

Edited by

Susan C. Cork and Roy W. Halliwell



First published 2019

Copyright © 5m Publishing 2019

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission of the copyright holder.

Published by 5M Publishing Ltd, Benchmark House, 8 Smithy Wood Drive, Sheffield, S35 1QN, UK Tel: +44 (0) 1234 81 81 80 www.5mpublishing.com

A Catalogue record for this book is available from the British Library

ISBN 9781789180459

Disclaimer

Every reasonable effort has been made to ensure that the material in this book is true, correct, complete and appropriate at the time of writing. Nevertheless, the publishers and the authors do not accept responsibility for any omission or error, or for any injury, damage, loss or financial consequences arising from use of the book. Views expressed are those of the authors and not of the editor or publisher.

Book layout by Keystroke, Neville Lodge, Tettenhall, Wolverhampton

Printed and bound in Wales by Gomer Press Ltd

Photos and illustrations by the authors unless otherwise indicated

Additional and supporting files are available on the book's website: http://fieldmanual.5mcreative.com/

This book is dedicated to the animal health and veterinary laboratory staff who work hard to provide support for farmers and communities that depend on their livestock.

Contents

| | Contributors | ix | | General bacteriology (Cork, Halliwell) | , |
|----|--|------|---|--|--------|
| | Foreword | xi | | Antimicrobial resistance (Cork, Liljebj | elke), |
| | Preface | xiii | | Virology (Abdul-Careem, Cork, | |
| | Acknowledgements | XV | | Abdul-Cader), Molecular Microbiolog | y |
| | List of figures, plates and tables | xvii | | (Abdul-Careem, Collins Emerson) | |
| I | LABORATORY AND EQUIPMENT | 1 | 5 | Haematology | 278 |
| | | | | Susan C. Cork and Roy Halliwell | |
| 1 | Setting up and using a laboratory | 1 | | | |
| | service | 3 | 6 | Serology and immunology | 300 |
| | Susan C. Cork, Roy Halliwell and Willy Schauwers | | | Susan C. Cork, M. Faizal Abdul-Caree and M. Sarjoon Abdul-Cader |) m |
| 2 | The selection, use, maintenance | | 7 | Clinical chemistry | 326 |
| | and quality control of laboratory equipment and supplies | 44 | | Susan C. Cork, Willy Schauwers and Roy Halliwell | |
| | Willy Schauwers | | | | |
| | | | 8 | Pathology/cytology | 363 |
| II | SPECIALTIES | 111 | | Susan C. Cork | |
| 3 | Parasitology | 113 | ш | SPECIAL TOPICS | 387 |
| | Susan C. Cork and Mani Leieune | | | | 007 |
| | | | 9 | Epidemiology | 389 |
| 4 | Microbiology | 198 | - | | |
| | | | | Susan C. Cork, Sylvia Checkley and | |
| | Susan C. Cork, Roy Halliwell, Karen | | | John Woodford | |
| | Lijebjeike, IVI. Faizal Abdul-Careem, | ine | | | |
| | IVI. Sarjoon Abdul-Cader and Julie Coll | INS | | | |
| | LINEISUN | | | | |

viii Contents

| 10 | Common clinical problems | 403 | IV | APPENDICES | 473 |
|----|--|------|----|--|-----|
| | Susan C. Cork | | 1 | Important zoonotic diseases | 475 |
| 11 | Wildlife health and disease surveillance | 420 | | Susan C. Cork | |
| | Matilde Tomaselli and Patricia Curry | | 2 | Necropsy guidelines | 494 |
| 12 | Antimicrobial resistance: | | | Samuel Sharpe | |
| | a threat to human and animal health | 433 | 3 | Some micro-organisms commonly isolated from animals | 534 |
| | Niamh Caffrey and Karen Tang | | | Susan C. Cork | |
| 13 | The World Organisation for Anima Health's core missions in relation | al | 4 | Examples of laboratory equipment and reagent suppliers | 539 |
| | to laboratories working in the veterinary domain | 442 | | Susan C. Cork | |
| | Jennifer Lasley | | 5 | Colibacillosis in poultry | 540 |
| 14 | Arthropod vectors and | 456 | | Karen Liljebjelke | |
| | ai tii opou-borne uiseases | -130 | | Glossary | 543 |
| | Regula Waeckerlin and Susan C. Cork | | | Index | 548 |

Contributors

- Dr M. Sarjoon Abdul-Cader, BVSc, MSc. Research Associate. Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada.
- Dr M. Faizal Abdul-Careem, BVSc (hons), MVM, PhD, DACPV, DACVM (virology) Associate Professor. Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada. email mfabdulc@ucalgary.ca
- **Dr Niamh Caffrey**, PhD, Research Associate, Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada.
- Dr Sylvia Checkley, DVM, PhD (Epidemiology) Associate Professor, Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada.
- Dr Susan C. Cork, BVSc, PhD, PG Dip. Public Policy, MRCVS. Professor, Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada. email sccork@ucalgary.ca
- **Dr Julie Collins Emerson**, BSc (Hons) PhD, Senior Research Officer, Infectious Disease Research Centre, Hopkirk Research Institute, IVABS (institute for Veterinary, Animal & Biomedical Sciences), New Zealand.
- **Dr Patricia Curry**, DVM, PhD, independent consultant, British Columbia, Canada.

- Roy Halliwell, MIBMS (specialist subject bacteriology). International laboratory consultant, Southport, United Kingdom.
- Jennifer Lasley, MPH. World Organisation for Animal Health, Sustainable Laboratory Initiatives, Programmes Department, OIE, Rue de Prony, Paris, France.
- Dr Karen Liljebjelke, BSc, MSc, DVM, PhD. Assistant Professor (Bacteriology), Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada. email kliljebj@ ucalgary.ca
- Dr Manigandan Lejeune, PhD, DipACVM (Parasitology) Director of Clinical Parasitology Population Medicine & Diagnostic Sciences College of Veterinary Medicine – Cornell University. email ml872@cornell.edu
- Willy Schauwers, BSc, international laboratory consultant, Gent, Belgium.
- Dr Samuel Sharpe, BSc (Hons), BVSc, (Anatomic Pathologist). Dip. ECVP, MRCVS. Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada.
- Dr Judit E.G. Smits. BSc, DVM, MVetSc, PhD, Professor (Wildlife & Ecotoxicology). Department of Ecosystem & Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada.,
- Dr Matilde Tomaselli, DVM, PhD, Department of Ecosystem & Public Health, Faculty of

Veterinary Medicine, University of Calgary, Alberta, Canada.

- **Dr Karen Tang**, MD, MSc, Assistant Professor, Cumming School of Medicine, University of Calgary, Alberta, Canada.
- Dr Regula Waeckerlin, DVM, PhD, Research Associate, Comparative Biology & Experimental

Medicine, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada.

Dr John Woodford, BVM, MSc, MRCVS. international veterinary consultant, France. email J.d.woodford@gmail.com

Foreword

As with the two preceding editions, this third edition of the *Veterinary Laboratory and Field Manual* is a book made to be used. Dr Susan Cork and her contributing colleagues, have drawn on their extensive experience in training, laboratory diagnostics and field development activities to produce a practical, informative and accessible guide to the organization and operation of district and regional level diagnostic laboratories.

Since the original publication of this book, the need for expanded veterinary diagnostic laboratory networks that extend into rural areas, has continued to grow. The ever increasing demand for animal protein and the concomitant increase in livestock populations, the emergence of new diseases and re-emergence of existing diseases, the expanded movement and trade of livestock, the increased contact of livestock and humans with wild animal species due to habitat incursions, and the growing risk of intentional disease introduction through bioterrorism, all underscore the need for improved disease surveillance and rapid diagnosis - efforts that require wellfunctioning district and regional laboratories as part of a national laboratory network. Early detection and early response are the fundamental elements of effective disease control and the ready availability of reliable basic laboratory services are essential in this regard.

The new edition maintains the wellorganized structure of past editions, starting with the most useful Part 1: Laboratory and equip-

ment. Chapter 1 focuses on setting up and using a laboratory service. This is followed by the practical and informative Chapter 2 on the selection, use and maintenance of laboratory equipment and supplies. Then, in Part II, in six subsequent chapters, the key diagnostic laboratory disciplines are addressed, including parasitology, microbiology, haematology, serology/immunology, clinical chemistry/toxicology, and pathology/cytology, each including the general underlying scientific principles, discussions of common diseases and procedures for sample collection and conduct of diagnostic tests. Part III then focuses on special topics including epidemiology, surveillance, common clinical presentations, wildlife health monitoring and disease surveillance, antimicrobial resistance, and arthropod vectors and related diseases. The book concludes with a range of appendices, which feature a review of common zoonotic diseases.

At various locations throughout the text, Dr Cork and colleagues have updated existing content and added some new topics. Among the new subjects considered are: training, information management and the value of laboratory networks; hazard assessment and risk management; audits for quality assurance; the benefits and limitations of penside tests; assessing diagnostic sensitivity and specificity for field tests; the applications, implementation and limitations of new technologies; and a chapter on the OIE's core missions in relation to laboratories working in the veterinary domain, contributed by the World Organisation for Animal Health (OIE).

The OIE is pleased to be associated with this new edition of the *Veterinary Laboratory and Field Manual.* The OIE's core strategic objectives, including risk management through transboundary disease control and international standard setting for the safe trade in animals and animal products, maintaining the global reporting system for animal diseases, and strengthening of national veterinary services, are all underpinned by the capacity and performance of national networks of veterinary diagnostic laboratories and the animal health investigation systems that deliver them quality samples. This newly revised manual can make an important contribution to ensuring the capacity and performance of those networks and therefore deserves a wide readership.

> Dr Matthew Stone Deputy Director General, International Standards and Science World Organisation for Animal Health (OIE) Paris, France June 2018

Preface

Donor agencies have made significant investments to support the improvement of veterinary extension services in low- to middle-income countries. This has often been accompanied by the establishment of laboratory networks. Despite a growth in both the number and capability of diagnostic laboratories, it remains evident that these services have often had a limited impact on the well-being of farmers in isolated rural communities or on the health and productivity of their livestock. Investment has often been focused on larger-scale agricultural enterprises and infrastructure and reporting systems at the national or regional level. Farmer and community contact remains predominantly with animal health extension services at the district level where resources are generally limited and laboratory support and facilities are poor.

With the origin of many emerging and reemerging human and livestock diseases often traced back to rural settings, where there is close interaction between humans, domestic animals and wildlife, it is essential that a broad One Health approach to disease detection is adopted and that the sustainable development and delivery of effective animal health extension services is supported. To achieve this, it is important to enhance collaborations and information exchange between and within government agencies. Early detection and rapid response to disease outbreaks in animals also requires access to a reliable and affordable veterinary diagnostic

service. The most important role of district and smaller regional veterinary diagnostic laboratories is to provide technical support for veterinary and animal health extension staff so that they can provide reliable animal health advice to the enduser, that is the farmer/community. This support includes the provision of technical advice, as well as diagnostic support, especially with regard to the submission of appropriate diagnostic samples and the interpretation of test results. The quality of the service provided depends on the level of training and experience of the staff, the availability of resources and on fostering good communication between field and laboratory based staff. In many cases the availability of training opportunities and suitable text books is restricted to staff based at well-resourced central and urban facilities.

Despite wider access to the internet and a proliferation of online learning opportunities, there remains a need for a general handbook to cover commonly used procedures for animal health and veterinary laboratory staff working in remote areas. This revised and updated handbook aims to provide an easy to follow summary of the laboratory techniques and sample collection guidelines that will be of practical value for routine diagnostic work in small regional and district veterinary laboratories. The new edition is supplemented by additional online material for those able to readily access the internet. The technology described is selected to emphasize the practical aspects of laboratory diagnosis and veterinary extension work rather than the theory. In addition to the basic laboratory disciplines of parasitology, microbiology, clinical chemistry, haematology and pathology, this updated edition includes some specialist sections on molecular diagnostics, syndromic surveillance in livestock, disease monitoring in wildlife and taking a One Health approach to antimicrobial resistance. There is also a special section on the role of the World Organization for Animal Health (OIE) in supporting veterinary diagnostic laboratories. For additional information, a bibliographic list is provided at the end of each chapter.

Acknowledgements

The first edition of this book was published in 2002 as a result of a collaboration between Roy Halliwell and myself. We began the project in 1997 while working on an EU-funded project to strengthen veterinary services in Bhutan. At that time, we realized that there was a need for a general diagnostic manual suitable for animal health extension staff and veterinary laboratory staff working together at the district level. The second edition was published a decade later and we included new and updated sections with the input of Willy Schauwers and other contributors. In this third edition we have engaged a number of additional subject experts: Dr Mani Lejeune, Chapter 3; Dr M. Faizal Abdul-Careem and M. Sarjoon Abdul-Cader, Chapter 4, Virology and Chapter 6; Dr Julie Collins Emerson, Chapter 4 molecular supplement; Dr Judit Smits, Chapter 7, Toxicology; Dr Sam Sharpe, Appendix 2 (Pathology SOPs) and Web content - necropsy

procedures, Pathology; Dr Niamh Caffrey and Dr Karen Tang MD, Chapter 12 (Antimicrobial resistance); Dr John Woodford, Chapter 9; Jennifer Lasley, Chapter 13 (OIE); Dr Matilde Tomaselli and Dr Patricia Curry, Chapter 11. We would like to thank all of the contributors for their engagement in this third edition and also the following for their comments on specific chapters: Barbara Martin (Chapter 1, Quality Management), Dr Karen Liljebjelke (Chapter 4, Bacteriology), Dr Cathy Monteith (Chapter 5, Haematology) and also the following for their assistance in the office, Robert Forsyth, Abir Bachir, Joy Punsalan and Katrine Maurer and the staff of 5M Publishing (Sarah Hulbert, Alessandro Pasini and Jeremy Toynbee) for editorial support.

> Dr Susan Catherine Cork Calgary, Alberta

List of figures, plates and tables

Figures

Figure 1.1 (a) Mobile laboratory with field team. (b) A district facility with mobile laboratory visiting from the regional veterinary facility. (c) Regional veterinary laboratory with small generator shed at the back of the building. (d) Regional veterinary laboratory parasitology section. (e) Regional veterinary laboratory microbiology staining sink. 6 Figure 1.2 Simplified ground plan for a regional laboratory. 8 Figure 1.3 An old oil drum or a metal rubbish bin can be used as an incinerator. 18 Figure 1.4 Diagram of a biological pit (longitudinal section). 19 Figure 1.5 Restraining an indigenous cow

for examination and blood sample collection, Khaling, Eastern Bhutan.

Figure 1.6 Collecting samples for the diagnosis of foot and mouth disease using a sterile swab.Figure 1.7 Field team restraining a young

cow for sample collection, Khaling, Eastern Bhutan. Figure 1.8 Restraint of a sheep for examination and trimming of the feet.

Figure 1.9 Samples which can be collected from a live animal. For more information see the relevant sections in Chapters 3–8. **Figure 1.10** Restraining a cow by means of a bull holder.

| | Figure 1.11 Restraining a cow by grasping the | |
|----|---|----|
| | nasal septum and one horn. | 23 |
| | Figure 1.12 Ropes can be used for casting | |
| ıg | cattle for examination especially where there | |
| | are no suitable handling facilities. | 23 |
| | Figure 1.13 Restraining a sheep by turning it | |
| γ | on its rump. | 24 |
| | Figure 1.14 Application of a twitch (a) to | |
| | control a mule (b and c). | 24 |
| 6 | Figure 1.15 The initial examination of a | |
| | 'downer' cow may be straightforward if the | |
| 8 | animal is resting comfortably but to determine | |
| | the cause of the problem it is important to | |
| 18 | obtain a full clinical history. | 25 |
| | Figure 1.16 Mouth gag for cattle (drinkwater | |
| 19 | gag), which is placed on one side of the jaw to | |
| | hold the mouth open. | 25 |
| | Figure 1.17 Using the ball of the finger (not | |
| 21 | the thumb) the pulse can be measured at the | |
| | middle coccygeal (tail) artery in the bovine. | 25 |
| | Figure 1.18a–d (a) The anatomy of the neck | |
| 21 | of the ruminant (topographical features). | |
| | (b, c and d) These photographs demonstrate | |
| | blood collection from the jugular vein of a dairy | |
| 22 | cow using a wide bore needle | |
| | and vacutainer. | 26 |
| 22 | Figure 1.19 Examination of the udder of a cow. | 27 |
| | Figure 1.20 A simple test for mastitis is the | |
| | California Mastitis Test which is available as | |
| 22 | a kit. | 27 |
| | Figure 1.21 Examination of breeding and | |
| 23 | neonatal animals. | 28 |

| Figure 1.22 Examination of a mule. | 28 | sun heat to warm up water, and solar PV | |
|---|----|--|----|
| Figure 1.23 If it is necessary to look into the | | systems, which convert sunlight directly into | |
| mouth of equine species or to rasp the teeth, | | electricity. | 49 |
| a gag such as the Haussman–Dunn gag | | Figure 2.9 Off-grid solar PV system | |
| (illustrated) may be required. | 28 | configuration. | 49 |
| Figure 1.24 Illustration of the area of the | | Figure 2.10 Sure Chill open refrigerator. | 54 |
| chest to listen to in the horse (a) or the cow | | Figure 2.11 -80°C freezer. | 54 |
| (b) if it is necessary to assess changes in | | Figure 2.12 Cold room 1. | 55 |
| respiratory sounds. | 29 | Figure 2.13 Cold room 2. | 55 |
| Figure 1.25 Lifting a forelimb of a horse may | | Figure 2.14 Simple cool box, to be used | |
| allow examination of the limb/foot and will | | with cool packs. | 56 |
| restrain the animal if it will not keep still. | 29 | Figure 2.15 Dry ice maker. | 56 |
| Figure 1.26 Using a small board to assist in | | Figure 2.16 Digital cool box, larger size, | |
| moving a pig to a suitable area for sample | | can be used in cars. | 57 |
| collection. | 30 | Figure 2.17 A maximum–minimum | |
| Figure 1.27 Collection of a blood sample from | | thermometer. | 58 |
| the ear vein of a pig using a 22 gauge needle | | Figure 2.18 Digital thermometer min/max | |
| and a syringe. | 30 | with external probe. | 58 |
| Figure 1.28 The approach to a post-mortem | | Figure 2.19 Datalogger with internal probe. | 59 |
| may differ depending on whether one or many | | Figure 2.20 Digital datalogger with multiple | |
| animals have died from a disease. | 31 | probes. | 59 |
| Figure 1.29 Sick animals may need to be | | Figure 2.21 Autoclaves can be used to sterilize | ; |
| euthanized for humane reasons or for disease | | equipment for simple surgical procedures as | |
| control. | 32 | well as laboratory ware, laboratory waste and | |
| Figure 1.30 Mule with load. | 32 | some instruments. | 60 |
| Figure 1.31 Application of a muzzle prior to | | Figure 2.22 Sterilization cycle in graphic | |
| examination of a dog. | 33 | format. | 61 |
| Figure 1.32 Sample collection box designed | | Figure 2.23 Autoclave tape before | |
| for carriage in a vehicle. | 35 | sterilization. | 62 |
| Figure 2.1 Voltage stabilizers can be used to | | Figure 2.24 Autoclave tape <i>after</i> sterilization. | 62 |
| protect laboratory equipment (refrigerators, | | Figure 2.25 Autoclave tape after sterilization | |
| freezers) against electricity power fluctuations. | 46 | (prominent black lines). | 62 |
| Figure 2.2 Surge protector. | 46 | Figure 2.26 Centrifuge. | 63 |
| Figure 2.3 UPS/no-break system with battery | | Figure 2.27 Different bucket sizes, to | |
| back-up (on right) for PCR-cycler (on left). | 46 | accommodate a range of specimen containers/ | |
| Figure 2.4 Equipment sensitive to power | | tubes. | 64 |
| breaks (like PCR cyclers) can be connected to | | Figure 2.28 Different bucket sizes. | 64 |
| a UPS/no-break battery power supply system. | 47 | Figure 2.29 Hand-operated centrifuge. | 65 |
| Figure 2.5 Solar PV refrigerator/freezer with | | Figure 2.30 Microhaematocrit centrifuge. | 65 |
| battery-system and inverter. | 47 | Figure 2.31 pH strip. | 67 |
| Figure 2.6 Solar PV panels. | 47 | Figure 2.32 pH meter. | 68 |
| Figure 2.7 Generator. | 48 | Figure 2.33 Incubator. | 69 |
| Figure 2.8 The two main types of solar power | | Figure 2.34 Oven. | 70 |
| systems: solar thermal systems, which trap | | Figure 2.35 Single pan balance. | 71 |
| | | | |

| Figure 2.36 | Analytical balance. | 71 |
|----------------|-----------------------------------|----|
| Figure 2.37 | Water bath. | 73 |
| Figure 2.38 | Deionizer. | 75 |
| Figure 2.39 | Manesty water distillation | |
| apparatus. | | 75 |
| Figure 2.40 | Air-displacement pipette. | 76 |
| Figure 2.41 | Positive displacement pipette. | 76 |
| Figure 2.42 | Micropipette. | 77 |
| Figure 2.43 | Dispenser. | 79 |
| Figure 2.44 | Semi-automatic photometer, | |
| Rayto RT-92 | 00 (wavelengths in the visible | |
| spectrum be | etween 330–800 nm, five standard | |
| filters, three | optional filters, flow cell. | 79 |
| Figure 2.45 | Diaspect Tm handheld | |
| haemoglobir | nometer. | 80 |
| Figure 2.46 | Parts of the microscope, | |
| Olympus CX | 43. | 81 |
| Figure 2.47 | Parts of the microscope, Leica | |
| DM4. | | 82 |
| Figure 2.48 | The microscopic field is divided | |
| according to | the plate of a clock. | 85 |
| Figure 2.49 | Systematic examination of the | |
| microscopic | field. | 86 |
| Figure 2.50 | Working principle of an oil | |
| immersion o | bjective. | 87 |
| Figure 2.51 | ELISA reader using filters. | 88 |
| Figure 2.52 | ELISA plate washing station. | 88 |
| Figure 2.53 | ELISA reader with a | |
| monochrom | ator (filters no longer needed). | 88 |
| Figure 2.54 | RT PCR cycler, safety cabinet and | |
| no-break-sys | stem. | 89 |
| Figure 2.55 | Sysmex pocH-100iV Diff | |
| haematology | / analyser. | 89 |
| Figure 2.56 | Sysmex pocH-100iV Diff screen. | 90 |
| Figure 2.57 | Standard glassware store | |
| containing fl | asks and glass bottles. | 91 |
| Figure 2.58 | Measuring volumes, reading the | |
| meniscus. | | 93 |
| Figure 2.59 | Glass reagent bottles. | 94 |
| Figure 2.60 | Beakers. | 94 |
| Figure 2.61 | Erlenmeyer flask. | 95 |
| Figure 2.62 | ivieasuring flask. | 95 |
| Figure 2.63 | ivieasuring cylinder. | 95 |
| Figure 2.64 | Pipette volumetric bunch. | 96 |

| Figure 2.65 Pipette volumetric. | 96 |
|---|-----|
| Figure 2.66 Pipette graduated. | 97 |
| Figure 2.67 Pipette volumetric close up. | 97 |
| Figure 2.68 Pipette filler. | 98 |
| Figure 2.69 Pipette filler electric. | 98 |
| Figure 2.70 Ultrasonic bath. | 103 |
| Figure 3.1 Various plastic bottles and bags | |
| may be suitable for collecting faecal samples. | 115 |
| Figure 3.2 The use of a plastic bag to protect | |
| hands from direct contact with faecal material | |
| if waterproof gloves are not available. | 116 |
| Figure 3.3 A temperature controlled | |
| centrifuge with an Eppendorf rotor. | 117 |
| Figure 3.4 Larval identification is difficult but | |
| some common species may be recognized by | |
| the characteristic length and shape of the tail | |
| as well as the number of cells in the intestinal | |
| tract. | 118 |
| Figure 3.5 Filling the chambers on a | |
| McMaster slide (A) for a worm egg count | |
| (see text). A fine tipped pipette (B) is used | |
| to fill the counting chamber(s) of the slide | |
| (C) with faecal suspension. | 120 |
| Figure 3.6 Flotation: (A) weighing 4 g of | |
| faecal material; (B) dilution of faecal material | |
| in water and filtering through a gauze pad into | |
| centrifuge tube; (C) filling of centrifuge tubes to | 0 |
| equal level; (D) diluting faecal pellet in Sheath | |
| solution after centinugation, (E) mining tubes | |
| enough to form meniscus, cover tubes with | |
| and transfer to labelled microscope slide | 121 |
| Figure 2.7 The Baermann equipment (A) used | 121 |
| for extracting lungworm and an illustration of a | |
| lungworm larva (B). A sieve (250 µm) is placed | |
| in the wide neck of a glass funnel held in a | |
| retort stand | 122 |
| Figure 3.8 The general topography of the | |
| bovine abdomen to show the location of the | |
| gut in situ. | 125 |
| Figure 3.9 Simple plan of the ruminant | |
| intestinal tract. | 125 |
| Figure 3.10 Detailed anatomical outline of | |
| the intestines of a cow. | 125 |
| | |

Figure 3.11 Relative size of adult worms which may be seen during a total worm count at post mortem examination. 126 Figure 3.12 Squash smear to show the appearance of a Trichinella spiralis larva developing in the muscle tissue of a pig (20×). 128 Figure 3.13 The life cycle of Trichinella spiralis is complex and because of their short lifespan, the adult worms are rarely found in natural infections. 129 Figure 3.14 Features used to assist in the identification of adult nematodes. 132 Figure 3.15 The microscopic appearance of parasite eggs commonly found in the faeces of pigs. 135 Figure 3.16 The microscopic appearance of parasite eggs commonly found in the faeces of horses and donkeys. 135 Figure 3.17 The microscopic appearance of parasite eggs commonly found in the faeces of ruminants. 136 Figure 3.18 The microscopic appearance of parasite eggs commonly found in the faeces of poultry. 136 Figure 3.19 The relative size of helminth ova, coccidial oocysts and artefacts. 137 Figure 3.20 (a) The microscopic appearance of parasite eggs commonly found in the faeces of carnivores. (b and c) Photomicrographs (40×) of the canine hookworm (Ancylostoma caninum) demonstrating the structure of the nematode mouth parts and the gut. 137 Figure 3.21 Basic gastrointestinal nematode life cvcle. 139 Figure 3.22 Life cycle of a nematode (Capillaria contorta) with an indirect life cycle. 140 Figure 3.23 Life cycle of a typical ruminant roundworm (for example, Haemonchus contortus). 141 Figure 3.24 Life cycle of the dog roundworm (Toxocara canis). 146 Figure 3.25 The life cycle of Dictyocaulus viviparous (bovine lungworm). 148

| Figure 3.26 Larval (cyst) forms of some | |
|--|-----|
| tapeworms. | 149 |
| Figure 3.27 Life cycle of Taenia solium and | |
| T. saginata. | 151 |
| Figure 3.28 Life cycle of Taenia multiceps, | |
| the tapeworm parasite that has a cyst stage | |
| in the brain causing 'gid' in the ruminant | |
| intermediate host. | 153 |
| Figure 3.29 Life cycle of the hydatid | |
| tapeworm (<i>Echinococcus granulosus</i>). | 155 |
| Figure 3.30 The life cycle of the liver fluke | |
| (Fasciola hepatica). | 157 |
| Figure 3.31 (a) The life cycle of <i>Eimeria</i> sp. | |
| Protozoal organisms of the genus <i>Eimera</i> or | |
| Isospora cause 'coccidiosis' in a number | |
| of species, in most cases the species of | |
| coccidia is host specific. (b) Photograph of | |
| <i>lsospora</i> sp. | 161 |
| Figure 3.32 The life cycle of Toxoplasma gondii. | 166 |
| Figure 3.33 Aborted foetus which was lost | |
| near to full term. | 167 |
| Figure 3.34 Life cycle of Sarcocytis sp. | 168 |
| Figure 3.35 Trypanosome (T) as seen in a | |
| Giemsa stained blood smear from a horse | |
| (1000× oil immersion). | 170 |
| Figure 3.36 Preparation of a blood film. | 173 |
| Figure 3.37 Climatic zones. | 181 |
| Figure 3.38 The general classification of | |
| arthropods of veterinary importance. | 185 |
| Figure 3.39 Morphological characteristics | |
| used to identify ticks. | 187 |
| Figure 3.40 Typical tick life cycle. | 188 |
| Figure 3.41 Life cycle of a three-host tick | |
| (for example, some <i>Rhipicephalus</i> sp., | |
| <i>lxodes</i> sp.). | 188 |
| Figure 3.42 Life cycle of a typical one-host | |
| tick (for example, <i>Rhipicephalus</i> [<i>Boophilus</i>] | |
| microplus, R. annulatus). | 189 |
| Figure 3.43 Photos of common | |
| ectoparasites. | 189 |
| Figure 3.44 Ventral view of two species of | |
| adult mites. | 190 |
| Figure 3.45 Stray dog with advanced | |
| sarcoptic mange. | 191 |
| · · · · · · · · · · · · · · · · · · · | |

| Figure 3.46 Dorsal view of two species of | | la |
|---|-----|----|
| adult louse (insects – three pairs of legs). | 192 | fa |
| Figure 3.47 Lateral view of a flea. (C) | | Fi |
| (Ctenocephalides sp.) cat and dog fleas. | 193 | te |
| Figure 3.48a Typical louse life cycle in which | | ba |
| there is no metamorphosis. | 195 | Fi |
| Figure 3.48b Typical fly life cycle | | te |
| characterized by metamorphosis. | 195 | th |
| Figure 3.49a The life cycle of the equine | | W |
| stomach bot (Gasterophilus intestinalis). | 196 | W |
| Figure 3.49b Horse stomach opened out to | | Fi |
| illustrate the appearance of the larvae of the | | th |
| horse bot fly (<i>Gasterophilus</i> sp.). | 196 | С |
| Figure 4.1 The relative size of a red cell, | | oł |
| a streptococcal bacterium, a chlamydial agent | | С |
| and an adenovirus. | 199 | Fi |
| Figure 4.2 The principal structures of a | | us |
| bacterial cell. | 200 | Fi |
| Figure 4.3 Scanning electron micrograph | | ar |
| (SEM) of a gram-negative bacterium | | Fi |
| (<i>Yersinia</i> sp.). | 201 | Fi |
| Figure 4.4 Microscopic appearance of | | te |
| rod shaped bacteria as seen using an oil | | Fi |
| immersion lens (1000×). | 202 | fu |
| Figure 4.5 Agar plates showing culture | | m |
| media and growth characteristics of colonies | | sp |
| of different microorganisms. | 209 | A |
| Figure 4.6 (a) Anaerobic jar with sealed lid | | Fi |
| (A) and a GasPak (B). (b) This is an example of | | of |
| a chamber used for growth of anaerobic | | Fi |
| bacteria. | 210 | st |
| Figure 4.7 Classical microbiology requires a | | le |
| wide range of reagents and relies on the | | Fi |
| technical expertise and experience of the | | ar |
| laboratory technician for the successful | | СС |
| culture and identification of disease causing | | ра |
| agents. | 211 | Fi |
| Figure 4.8 Initial 'streaking' of a culture plate | | vi |
| and preparation of a subculture (or 'purity' | | Fi |
| plate). | 215 | W |
| Figure 4.9 Classification chart according to | | di |
| staining reaction and cellular morphology of | | e |
| common bacteria of veterinary importance. | 216 | Fi |
| Figure 4.10 Sink in a small microbiology | | W |

| laboratory at a district veterinary diagnostic | |
|--|-----|
| facility. | 217 |
| Figure 4.11a and b Primary biochemical | |
| tests for (a) Gram +ve and (b) Gram –ve | |
| bacteria. | 221 |
| Figure 4.11c Simple biochemical screening | |
| test (TSI slopes) can be used to determine | |
| the species of selected bacteria during survey | |
| work but in most cases a series of 20-30 tests | 3 |
| will be required. | 222 |
| Figure 4.11d API strip card used to illustrate | |
| the typical biochemical reactions of 'type' | |
| cultures representative of bacterial species | |
| obtained from the American Type Culture | |
| Collection. | 224 |
| Figure 4.12 The dilution technique can be | |
| used to count bacteria (see text). | 225 |
| Figure 4.13a Factors affecting the choice of | |
| an antibacterial drug. | 226 |
| Figure 4.13b Antibiotic sensitivity testing. | 230 |
| Figure 4.13c and d Antibiotic sensitivity | |
| testing plates. | 230 |
| Figure 4.14a The morphology of yeasts and | |
| fungi; this figure demonstrates some of the | |
| morphological features of yeasts and the | |
| species of fungi (<i>Penicillium</i> spp. and | |
| Aspergillus sp.). | 235 |
| Figure 4.14b Diagrammatic representation | |
| of some fungal species. | 236 |
| Figure 4.15 Steps in viral disease diagnosis | |
| starting from the evaluation of clinical signs | |
| leading to laboratory confirmation. | 241 |
| Figure 4.16 Modern laboratory assays | |
| are available targeting various structural | |
| components of the enveloped and naked viral | |
| particles. | 241 |
| Figure 4.17 Identification and isolation of | |
| viruses from clinical samples. | 242 |
| Figure 4.18a Canine airway epithelium | |
| with intracytoplasmic inclusions of canine | |
| distemper virus (the arrow indicates | |
| eosinophilic intracytoplasmic inclusion bodies). | 244 |
| Figure 4.18b Trachea of a chicken infected | |
| with infectious larvngotracheitis virus with | |
| | |

| multinucleated syncytial cell formation | | the prevention of product carry over or cross | |
|--|-----|---|-----|
| (the arrow indicates eosinophilic | | contamination. | 263 |
| intracytoplasmic inclusion bodies). | 244 | Figure 4.31 (a) A microarray reader is | |
| Figure 4.19 Immunohistochemistry staining | | necessary to scan the amount of fluorescent | |
| can be performed in frozen sections or | | labelled probes hybridize with the target nuclei | ic |
| formalized sections to visualize viral antigens. | 244 | acid in the sample. (b) Scanned array image | |
| Figure 4.20 Transmission electron microscopy | / | showing positive (yellow) and negative results | |
| (TEM) imaging of macrophages infected with | | (black). | 264 |
| infectious bronchitis virus (corona virus). | 245 | Figure 4.32 (a) Thermocycler used in | |
| Figure 4.21 Image captured under | | conventional PCR technique. (b) Loading of | |
| fluorescent microscope following staining of | | PCR products onto an agarose gel for | |
| trachea infected with infectious bronchitis viru | S | electrophoresis. (c) Gel electrophoresis of | |
| demonstrating nuclear antigen of the virus | | conventional PCR products. | 270 |
| (reddish colour). | 245 | Figure 4.33 Real-time PCR amplification | |
| Figure 4.22 Essential equipment required | | curves and the melting peaks. | 271 |
| to establish a laboratory with cell culture | | Figure 4.34 (a) Isothermal amplification | |
| facility. | 246 | requires only a simple heating block to | |
| Figure 4.23 In plaque assay the virus | | maintain constant temperature up to an hour. | |
| inoculum is ten-fold serially diluted in | | (b) LAMP isothermal amplification products | |
| phosphate buffered saline and inoculated into | | can be visualized using naked eye due to the | |
| the monolayer of cells which are permissive | | accumulation of PCR by product magnesium | |
| to the inoculating virus. | 247 | pyrophospahte (cloudy) or colour change | |
| Figure 4.24 Infectious laryngotracheitis | | using SYBR green. | 272 |
| virus (herpesvirus) replicates and produces | | Figure 4.35 (a) Modern sequencing | |
| cytopathic effects on monolayer of leghorn | | equipment used for nucleotide sequencing. | |
| male hepatoma (LMH) cells. | 248 | (b) A representative of sequencing output. | 273 |
| Figure 4.25 (a) Egg incubator is an essential | | Figure 4.36 Phylogenetic Tree of a total of | |
| component of a virology laboratory. (b) Pock | | over 180 partial σ C gene sequences of avian | |
| lesions on the CAM of embryonated chicken | | reovirus. | 274 |
| egg two days of inoculation. (c) The embryo | | Figure 4.37 Next-generation sequencing | |
| on the left is the uninfected control. | 248 | equipment used for rapid sequencing of | |
| Figure 4.26 Egg inoculation routes using an | | whole genomes or metagenomes. | 275 |
| embryonated chicken egg (at 9–11 days of | | Figure 4.38 MinIon sequencing device, | |
| incubation). | 249 | (Oxford Nanopore Technologies). | 276 |
| Figure 4.27 In SN assay, the unknown serum | | Figure 5.1 A diagrammatic representation | |
| sample is two-fold serially diluted and titrated | | of the appearance of blood cells in stained | |
| against a known quantity of virus. | 251 | blood films (not to scale). | 280 |
| Figure 4.28 Western blot assay | | Figure 5.2 (a) Blood smear modified from | |
| demonstrating pathogenic prion proteins, | | Pratt (1997). (b) Samples 1–6 are blood | |
| PrPsc, in brain homogenates. | 253 | smears, sample 18 is a tissue smear. | 283 |
| Figure 4.29 Influenza A and B viruses can | | Figure 5.3 Battlement counting method. | 286 |
| be differentiated based on rapid antigen | | Figure 5.4 (a) Filling the counting chamber | |
| ELISA test conducted on a membrane. | 262 | using a Pasteur pipette. (b) Improved Neubauer | |
| Figure 4.30 PCR work stations are used for | | ruling for a blood counting chamber. | 287 |

| Figure 5.5 (a) Microhaematocrit reader. | |
|---|-----|
| (b) Microhaematocrit centrifuge. | 293 |
| Figure 5.6 Blood smear from a cow | |
| which later died following fever, haematuria | |
| and weight loss over a period of several | |
| days. | 295 |
| Figure 5.7 Poor quality bovine blood smear | |
| stained with Giemsa 100× oil immersion. | 295 |
| Figure 5.8 Equine blood smear viewed under | |
| oil immersion (Diff Quick 1000×) illustrating | |
| numerous polymorph neutrophils (N), an | |
| eosinophil (E) and a basophil (B). | 296 |
| Figure 5.9 Equine blood smear viewed | |
| under oil immersion 1000× illustrating | |
| granulocytes (E and N) and agranulocytes | |
| (M and L). (E) Eosinophil, (N) polymorph | |
| neutrophil. | 296 |
| Figure 5.10 (a) Canine band neutrophil | |
| (immature PMN). (b) Feline eosinophil. | |
| Note reddish granules in the cytoplasm and | |
| segmented nucleus. (c) Feline basophil. | |
| (d) Feline blood smear showing rouleaux | |
| formation. | 297 |
| Figure 6.1 (a) Immune response generated | |
| following exposure to a pathogen can be | |
| innate (non-specific) response and adaptive | |
| (specific) response. (b) Innate immune response | se |
| leads to antigen presentation and subsequent | |
| development of adaptive response. | 301 |
| Figure 6.2(a) Diagrammatic representation of | |
| some aspects of phagocytosis, which is part | |
| of the innate immune response. | 302 |
| Figure 6.2(b) Essential features of the | |
| immune response. | 303 |
| Figure 6.3 A simple chart outlining the cells | |
| involved in the specific immune response | |
| which consists of (1) humoral response and | |
| (2) cell-mediated response. | 304 |
| Figure 6.4(a) The structures of classes of | |
| antibodies. | 306 |
| Figure 6.4(b) Antigen-antibody binding - | |
| precipitation. | 307 |
| Figure 6.5 Serum antibody concentrations | |
| following primary and secondary infections | 200 |
| following primary and secondary infections. | 300 |

| Figure 6.6 Diagrammatic representation of | |
|--|-----|
| what can be seen in the slide agglutination | |
| test. | 311 |
| Figure 6.7(a) Tube agglutination test with | |
| doubling dilutions beginning at 1 in 5. | 312 |
| Figure 6.7(b) The quantitative precipitin test. | 313 |
| Figure 6.8(a) Typical layout used for an | |
| 'Ochtelony' diffusion test. | 315 |
| Figure 6.8(b) Agar gel immunodiffusion test. | 315 |
| Figure 6.8(c) Agar gel immunodiffusion is | |
| a qualitative test is used for the detection of | |
| antibodies against influenza A virus routinely. | 316 |
| Figure 6.9 (a) Photograph of a plate used to | |
| perform the HI test (note that this is not the | |
| same test plate as that shown in Figure 6.9b). | 317 |
| Figure 6.10 Principle of the CFT. | 319 |
| Figure 6.11 Fluorescent antibody technique/ | |
| immunofluorescence. | 320 |
| Figure 6.12 (a) Immunocytochemical | |
| staining of a section of liver (20×) from a bird | |
| that died following infection with Yersinia | |
| pseudotuberculosis. (b) Immunocytochemical | |
| staining of a section of intestine from a dog | |
| which died following infection with parvo viral | |
| infection. | 321 |
| Figure 6.13 (a) ELISA technique for | |
| measuring the amount of antigen in a test | |
| sample (indirect method). | |
| (b) ELISA technique for measuring the amount | |
| of antibody in a test serum (indirect method). | 323 |
| Figure 7.1 Chemstrip® uG/K are test | |
| strips used in human medicine for the semi- | |
| quantitative determination of glucose in urine | |
| and for the detection of ketone bodies | |
| (for example, acetone) in urine. | 331 |
| Figure 7.2 The principle of wavelength. | 332 |
| Figure 7.3 The electromagnetic spectrum. | 333 |
| Figure 7.4 The visible part of the | |
| electromagnetic spectrum. | 333 |
| Figure 7.5 Light reflected, absorbed and | |
| transmitted when it falls on a coloured | |
| solution. | 334 |
| Figure 7.6 Calibration graph showing the | |
| potential linear or exponential relationship | |

| between absorbance readings and | | Figure 8.6 Respiratory system of the cow. | 372 |
|---|-----|---|-----|
| concentration. | 334 | Figure 8.7 Schematic representation of the | |
| Figure 7.7 Plotting values for quality control | | bovine abdomen showing the location of the | |
| (QC). | 335 | urogenital organs of a cow. | 373 |
| Figure 7.8 Schematic representation of the | | Figure 8.8 Superficial lymph flow of the | |
| distribution of results for a serum assay | | bovine. | 374 |
| ('normal distribution'). | 336 | Figure 8.9 Schematic view of the bovine | |
| Figure 7.9 Schematic representation of the | | central nervous system. | 375 |
| distribution of results for a laboratory test | | Figure 8.10 Goat skull. | 376 |
| measuring a substance which has a 'skewed' | | Figure 8.11 Skeleton of a cow. | 377 |
| range of values. | 337 | Figure 8.12 Anatomy of the lower limb of | |
| Figure 7.10 Atomic structure of carbon. | 341 | the horse (typical of an 'odd toed' ungulate). | 378 |
| Figure 7.11 Reading the level of a fluid | | Figure 8.13 A schematic view of the | |
| column (meniscus). | 343 | topographical anatomy of an elephant to show | , |
| Figure 7.12 Standard dilution technique | | that although many wild or exotic species may | / |
| for a 1 : 10 serial dilution and a 1 : 2 serial | | look quite different to domestic ruminants the | |
| dilution. | 346 | general anatomy is similar. | 379 |
| Figure 7.13 Microscopic examination of | | Figure 8.14 There are often a lot of deaths | |
| urine sediment (stylized and not to scale). | 353 | at lambing time but the presence of the | |
| Figure 7.14 (a) Organophosphate or | | occasional deformed lamb may not | |
| carbamate poisoning can be diagnosed from | | necessarily indicate that there is a disease | |
| a serum sample of the patient using basic | | present. | 382 |
| laboratory supplies and reagents, (b) glass | | Figure 8.15 This old ewe was found in a bog | |
| slides with positive and negative serum | | shortly after lambing. | 382 |
| samples are shown in the insert. | 356 | Figure 8.16 This old ewe has started to lose | |
| Figure 7.15 (a) A formalin fixed bovine brain | | her wool after recovering from ketosis | |
| with polioencephalomalacia in the grey matter | , | (twin lamb disease). | 382 |
| has subtle, sunken yellowish necrotic areas, | | Figure 8.17 Laboratory technician setting | |
| (b) which show dramatic fluorescence in a | | out to perform a post-mortem. | 383 |
| dark room using UV light. | 357 | Figure 8.18 If a post-mortem is carried out | |
| Figure 7.16 Hind limbs from beef cattle | | under field conditions it is important to explain | |
| suffering from severe ergotamine toxicity, | | each step of the process to the farmer. | 383 |
| show the classic lesions with the lost blood | | Figure 8.19 Skin rash seen in a pig with | |
| supply resulting in tissue death, with hooves | | Erysipelothrix insidiosa sp. (now E. rhusiopathia | ne) |
| and feet falling off. | 358 | infection. | 383 |
| Figure 8.1 Containers used for pathological | | Figure 8.20 This photograph illustrates a | |
| specimens. | 364 | common set up for image analysis where | |
| Figure 8.2 Bovine internal organs (bull), | | histology images can be viewed directly on | |
| right side view. | 367 | a computer screen and specific areas can be | |
| Figure 8.3 Bovine internal organs (cow), | | measured and mapped based on differences | |
| left side view. | 368 | in staining intensity or other differential | |
| Figure 8.4 A schematic representation of | | markers. | 384 |
| the topographical anatomy of a bird. | 368 | Figure 8.21 Histology section H&E 20× of | |
| Figure 8.5 Bovine cardiovascular system. | 371 | a normal chicken (<i>Gallus gallus</i>) lung. | 384 |
| | | | |

| Figure 8.22 Histology section H&E 20× of a | two settlements established on the island, | | | |
|--|--|---|-----|--|
| bird intestine (chicken, Gallus gallus) illustrating | | Iqaluktutiaq, or Cambridge Bay, Nunavut | | |
| haemorrhage secondary to a bacterial | | (study area) and Ulukhaktok, Northwest | | |
| infection. | 384 | Territories (approximately 1700 and 400 | | |
| Figure 8.23 Histology section of a bird liver | | people, respectively). | 426 | |
| 40× stained with Perls' Prussian Blue iron | | Figure 11.2 Schematic representation | | |
| stain to illustrate the presence of iron stored | | of the participatory muskox health | | |
| as hemosiderin in hepatocytes and Kupffer | | surveillance programme developed in the | | |
| (macrophage) cells. | 385 | community of Cambridge Bay in the Canadian | | |
| Figure 8.24 Illustration of gross necropsy on | | Arctic. | 427 | |
| a freshly dead aviary bird (parakeet) illustrating | | Figure 11.3 Schematic representation of the | | |
| an enlarged and discoloured liver with several | | proportional piling technique used in the group |) | |
| abscesses. | 385 | interviews performed with participants of the | | |
| Figure 8.25 The use of immune- | | community of Cambridge Bay (Victoria Island, | | |
| histochemistry to identify lesions caused by | | Nunavut). | 427 | |
| Yersinia pseudotuberculosis (serotype 3) in | | Figure 11.4 (A) A hunted muskox cow | | |
| the liver of the case illustrated in Figure 8.24. | 385 | with a sampling kit that will be used by the | | |
| Figure 8.26 Histology section of a wild bird | | hunter to collect a set of biological samples | | |
| liver (New Zealand kokako [Callaeas cinereal]) | | when butchering the carcass. (B) The core | | |
| 10× stained with Perls' Prussian Blue iron | | set of samples collected using the sampling | | |
| stain to illustrate the presence of excess | | kit: blood-saturated filter-paper strips, | | |
| stored iron. | 386 | faeces, left metatarsus (or left hind leg), | | |
| Figure 9.1 Intensive commercial dairy farm. | 394 | and a piece of skin with hair from the | | |
| Figure 9.2 North western Ethiopia – mixed | | rump. | 428 | |
| farming system using horses to plough arable | | Figure 11.5 Examples of field disease | | |
| land. | 395 | investigations. | 429 | |
| Figure 9.3 Northern Kenya, Samburu | | Figure 12.1 Potential mechanisms of | | |
| pastoralists, extensive herding of cattle, | | transmission of antibiotic resistance from | | |
| sheep and goats. | 395 | animals to humans. | 434 | |
| Figure 9.4 Veterinarian working with a group | | Figure 12.2 The One Health approach | | |
| of community-based animal health workers an | d | considers the interactions in health among | | |
| livestock keepers using PDS tools during the | | humans, animals, and the environment. | 435 | |
| active surveillance for Rinderpest in Kenya in | | Figure 12.3 OIE list of antimicrobials of | | |
| 2001. | 396 | veterinary importance in 2015. | 436 | |
| Figure 9.5 Veterinarian training veterinary | | Figure 12.4 Practices to improve biosecurity | | |
| paraprofessionals to perform a post-mortem | | on farms. | 438 | |
| examination and how to collect and handle | | Figure 13.1 OIE's regional and subregional | | |
| appropriate laboratory specimens, Punjab, | | offices around the world. | 442 | |
| Pakistan. | 397 | Figure 13.2 The veterinary domain: | | |
| Figure 10.1 Some common causes of abortio | n | veterinary services. | 444 | |
| in pigs and the time of gestation at which | | Figure 13.3 OIE's International Standards: | | |
| abortions typically occur. | 407 | Terrestrial Animal Health Code, Aquatic Anima | I | |
| Figure 11.1 Map of Victoria Island in the | | Health Code, Manual of Diagnostic Tests and | | |
| Canadian Arctic Archipelago showing the only | | Vaccines for Terrestrial Animals and Manual | | |
| | | | | |

of Diagnostic Tests and Vaccines for Aquatic Animals. 445 Figure 13.4 OIE Performance of Veterinary Services (PVS) tool. 451 Figure 13.5 PVS Gap Analysis (Costing Tool). 453 Figure 13.6 PVS Pathway Laboratory Tool. 454 Figure 14.1 Carbon dioxide mosquito trap components and trap in the field. 461 Figure 14.2 Ecological considerations for the sampling of ticks. 470 Figure A2.1 Bovine left ventricular papillary muscle. 506 Figure A2.2 Bovine cerebral cortex. 511 Figure A2.3 Removing the brain. 512 Figure A2.4 Removing the brain. 512 Figure A2.5 Abdomen, cat. 520 Figure A2.6 Heart, bovine. 520 Figure A2.7a Thorax, bovine. 521 Figure A2.7b Cut surface, lung from Figure A2.7a. 521 Figure A2.8 Liver, sheep. 522 Figure A2.9 Urinary tract, dog. 522 Figure A2.10 Liver, sheep. 523 Figure A2.11 Kidney, bovine. 524 Figure A2.12 Pluck, sheep, the trachea is on the left side of the picture (†). 525 Figure A2.13 Aorta, sheep. 525 Figure A2.14 Abdomen, dog. 526 526 Figure A2.15 Liver, sheep. Figure A2.16 Thoracic wall, cat. 527 Figure A2.17 Pluck, cat. 527 527 Figure A2.18 Liver, bovine. Figure A2.19 Abomasum, calf. 528 Figure A5.1 Diagnostic scheme for common veterinary bacterial pathogens. 541 Figure A5.2 A basic microbiology and molecular biology diagnostic scheme for identifying and characterizing bacteria. 541

Plates (positioned between pages 386 and 387)

| Plate 1 Modified nematode larval culture. |
|---|
| Plate 2a Nematode eggs. |
| Plate 2b Nematode eggs and larvae. |
| Plate 3 Trematode and cestode eggs. |
| Plate 4 Protozoans. |
| Plate 5 Faecal flotation technique. |
| Plate 6 Modified Baermann technique. |
| Plate 7 Common ectoparasites. |
| Plate 8 Agar plates showing culture media and |
| growth characteristics of colonies of different |
| microorganisms. |
| Plate 9 Simple biochemical screening test (e.g |
| Triple Sugar Iron (TSI) agar slopes) can be used to |
| determine the species of selected bacteria during |
| survey work but in most cases a series of 20–30 |
| tests will be required. |
| Plate 10 API strip card used to illustrate the |
| typical biochemical reactions of 'type' cultures |
| representative of bacterial species obtained from |
| the American Type Culture Collection. |
| Plate 11 Canine airway epithelium with |
| intracytoplasmic inclusions of canine distemper |
| virus. |
| Plate 12 Trachea of a chicken infected |
| with infectious laryngotracheitis virus with |
| multinucleated syncytial cell formation. |
| Plate 13a & b Immunohistochemistry staining |
| can be performed in frozen sections or formalized |
| sections to visualize viral antigens. |
| Plate 14 Image captured under fluorescent |
| microscope following staining of trachea infected |
| with infectious bronchitis virus demonstrating |
| nuclear antigen of the virus. |
| Plate 15 Avian influenza virus titration has been |
| done in Madin-Darby Canine Kidney (MDCK) cells. |
| Plate 16 In serum neutralization assay, the |
| unknown serum sample is two-fold serially |
| diluted and titrated against a known quantity of |
| virus. |
| Plate 17 Seanned array image showing positive |

Plate 17 Scanned array image showing positive (yellow) and negative results (black).

Plate 18 Real-time PCR amplification curves and the melting peaks.

Plate 19 LAMP isothermal amplification products can be visualized using naked eye due to the accumulation of PCR by product magnesium pyrophospahte (cloudy) or colour change using SYBR green.

Plate 20 A representative of sequencing output. **Plate 21** Phylogenetic Tree of a total of over 180 partial σC gene sequences of avian reovirus.

Plate 22a Blood smear from a cow which later died following fever, haematuria and weight loss over a period of several days. Giemsa 20x magnification.

Plate 22b Poor quality bovine blood smear stained with Giemsa 100× oil immersion.

Plate 23 Equine blood smear viewed under oil immersion (Diff Quick 1000×) illustrating numerous polymorph neutrophils (N), an eosinophil (E) and a basophil (B). Diff Quick[™] stain. RBC are also present.

Plate 24 Equine blood smear viewed under oil immersion 1000× illustrating granulocytes (E an N) and agranulocytes (M and L).

Plate 25 Canine band neutrophil (immature PMN).

Plate 26 Feline eosinophil.

Plate 27 Feline basophil.

Plate 28 Feline blood smear showing rouleaux formation.

Plate 29 Immune response generated following exposure to a pathogen can be innate (non-specific) response and adaptive (specific) response.

Plate 30 Serum antibody concentrations following primary and secondary infections.

Plate 31 Photograph of a plate used to perform the haemagglutination inhibition test.

Plate 32 Diagrammatic representation of the haemagglutination inhibition test.

Plate 33 Glass slides with organophosphate positive and negative serum samples are shown in the insert.

Plate 34 A formalin fixed bovine brain with polioencephalomalacia in the grey matter.

Plate 35 Section of brain under ultraviolet light. Fluorescent sections stand out.

Plate 36 There are often a lot of deaths at lambing time but the presence of the occasional deformed lamb may not necessarily indicate that there is an infectious disease present. Toxins can also cause deformities.

Plate 37 Skin rash seen in a pig with *Erysipelothrix insidiosa* sp.

Plate 38 Histology section H&E 20× of a normal chicken (*Gallus gallus*) lung.

Plate 39 Histology section H&E 20× of a bird intestine (chicken, *Gallus gallus*) illustrating haemorrhage secondary to a bacterial infection.

Plate 40 Histology section of a bird liver 40× stained with Perl's Prussian Blue iron stain to illustrate the presence of iron stored as hemosiderin in hepatocytes and Kupffer (macrophage) cells.

Plate 41 Illustration of gross necropsy on a freshly dead aviary bird (parakeet) illustrating an enlarged and discoloured liver with several abscesses.

Plate 42 The use of immune-histochemistry to identify lesions caused by *Yersinia pseudotuberculosis* (serotype 3) in the liver of the case illustrated in Plate 41.

Plate 43 Histology section of a wild bird liver (New Zealand kokako [*Callaeas cinereal*]) 10× stained with Perl's Prussian Blue iron stain to illustrate the presence of excess stored iron.

Plate 44 (A) A hunted muskox cow with a sampling kit that will be used by the hunter to collect a set of biological samples when butchering the carcass. (B) The core set of samples collected using the sampling kit: blood-saturated filter-paper strips, faeces, left metatarsus (or left hind leg), and a piece of skin with hair from the rump.

Plate 45 Bovine cerebral cortex.

Plate 46 Abdomen, cat. Acute inflammation. Plate 47 Thorax, bovine with chronic suppurative bronchopneumonia.

Plate 48 Liver, sheep. Necrosis.

Plate 49 Liver, sheep. Chronic fascioliasis
(Fasciola hepatica).
Plate 50 Kidney, bovine. Autolysis.
Plate 51 Pluck, sheep, the trachea is on the left side of the picture (†).
Plate 52 Abdomen, dog. Bile imbibition.
Plate 53 Liver, sheep. Pseudomelanosis.
Plate 54 Liver, bovine. Post-mortem emphysema.

Tables

| Table 1.1 Handling of common laboratory | |
|---|-----|
| wastes. | 17 |
| Table 1.2 Normal clinical parameters for | |
| various domestic animals. | 20 |
| Table 1.3 Suggested materials for a simple | |
| sample collection kit. | 36 |
| Table 1.4 Suggested materials for a simple | |
| post-mortem kit. | 37 |
| Table 2.1 Tools required for a one- or | |
| two-person laboratory workshop. | 51 |
| Table 2.2 Example of an annual equipment | |
| maintenance and service plan. | 52 |
| Table 2.3 Basic adjustments of the | |
| microscope. | 84 |
| Table 2.4 Plastic ware properties: chemical | |
| resistance and possibility to autoclave. | 92 |
| Table 2.5 Classes of active substances | |
| used in chemical disinfectants. | 105 |
| Table 3.1 Approximate size and location of | |
| common helminth parasites. | 126 |
| Table 3.2 Examples of tapeworm species. | 127 |
| Table 3.3 A guide to the significance of | |
| faecal egg count (EPG – eggs per gram) | |
| in faecal samples collected from sheep and | |
| cattle. | 131 |
| Table 3.4 The classification of nematodes | |
| of veterinary importance. | 133 |
| Table 3.5 Final and intermediate hosts of | |
| common tapeworms. | 152 |
| Table 3.6a Overview of the classification | |
| of some protozoa of veterinary significance. | 162 |

| | Table 3.6b Classification of Rickettsiae. | 163 |
|---|---|-----|
| | Table 3.7 Some coccidial protozoa and their | |
| | hosts. | 164 |
| | Table 3.8 Host relationships for some | |
| | species of Sarcocysts. | 168 |
| | Table 3.9 The classification of ticks of | |
| | veterinary importance and the diseases they | |
| | transmit. | 183 |
| | Table 3.10 The classification of mites of | |
| | veterinary importance. | 184 |
| | Table 3.11 Some insects of veterinary | |
| | importance and the diseases they transmit. | 194 |
| | Table 4.1 The staining characteristics of | |
| | some bacteria of veterinary importance. | 205 |
| 7 | Table 4.2a Media used for the isolation | |
| | and identification of common bacterial | |
| 0 | pathogens. | 210 |
| | Table 4.2b Cultural characteristics of some | |
| 6 | common bacteria found in veterinary | |
| | medicine. | 212 |
| 7 | Table 4.3 A summary of available | |
| | miniaturized biochemical test kits. | 223 |
| 1 | Table 4.4a Bacterial mechanisms to | |
| | enhance survival in the host. | 231 |
| 2 | Table 4.4b Mode of Action of some | |
| | commonly used antibacterial drugs. | 232 |
| 4 | Table 4.5 Isolation and identification of | |
| | some viruses of veterinary importance. | 243 |
| 2 | Table 4.6 Examples of commercial 'kit tests' | |
| | available for the diagnosis of viral infections | |
| 5 | in livestock. | 254 |
| | Table 4.7a The classification of some DNA | |
| 6 | viruses of veterinary importance. | 265 |
| 7 | Table 4.7b The classification of some RNA | |
| | viruses of veterinary importance. | 266 |
| | Table 5.1 Components of the haematopoietic | |
| | system and their function. | 279 |
| 1 | Table 5.2 Blood collection sites in different | |
| | species of animal. | 282 |
| 3 | Table 5.3 Characteristics of blood cells in | |
| _ | different species of animal. | 289 |
| 2 | Table 5.4 Normal haematological | |
| _ | parameters for livestock and companion | |
| 2 | anımals. | 290 |

| Table 5.5 Simplified explanation of various | |
|---|-----|
| changes to white cell populations in blood | |
| samples. | 298 |
| Table 6.1 Extent of reaction for IgG | |
| compared to that of IgM. | 311 |
| Table 7.1 Summary chart for blood sample | |
| collection and recommendations for specific | |
| biochemical tests. | 327 |
| Table 7.2 Normal* serum values for | |
| biochemical parameters in common domestic | |
| species. | 329 |
| Table 7.3 Conversion units for biochemical | |
| parameters. | 330 |
| Table 7.4 International atomic weights of | |
| selected elements. | 341 |
| Table 7.5 Interpretation of the bilirubin test. | 348 |
| Table 7.6 Preparation of reagents. | 349 |
| Table 7.7 Diagnostic samples to submit for | |
| various suspected toxicants. | 359 |
| Table 9.1 Examples of common clinical | |
| disease syndromes (ruminants) and | |
| corresponding possible notifiable diseases or | |
| other differential diagnoses and the laboratory | |
| samples required to make a definitive | |
| laboratory diagnosis (zoonoses in grey). | 401 |
| Table 10.1 Gestation periods of common | |
| domestic species. These figures are | |
| estimates for common breeds but there can | |
| be a variation. | 405 |

| Table 10.2 Some infectious causes of | |
|--|-----|
| abortion in cattle. | 406 |
| Table 10.3 Some causes of diarrhoea in | |
| horses. | 409 |
| Table 10.4 Some causes of diarrhoea in | |
| cattle. | 410 |
| Table 10.5 Some causes of diarrhoea in pigs. | 410 |
| Table 10.6 Summary of some diseases to be | |
| considered as possible causes of red urine in | |
| cattle. | 412 |
| Table 10.7 Terms used to identify skin | |
| changes. | 414 |
| Table 10.8 Common causes of hair loss and | |
| skin disease. | 415 |
| Table 10.9 Causes of weight loss and failure | |
| to gain weight. | 417 |
| Table 10.10 Summary of some common | |
| causes of neurological disturbance in | |
| livestock. | 419 |
| Table 11.1 Examples of diseases that can | |
| be transmitted between wild and domestic | |
| animal populations. | 421 |
| Table 14.1 Important mosquito-borne | |
| viruses. | 457 |
| Table 14.2 Important tick-borne pathogens. | 462 |
| Table A1.1 Summary of the source and | |
| clinical signs associated with some common | |
| zoonotic diseases. | 476 |
| Table A3.1 | 535 |

PART I

LABORATORY AND EQUIPMENT

chapter 1

Setting up and using a laboratory service

Susan C. Cork, Roy Halliwell and Willy Schauwers

1.1 The role of the veterinary laboratory network within animal health extension services

Most veterinary laboratory networks consist of a central research and/or referral laboratory and a number of regional laboratories, which are less well equipped than the central facility but are able to support most of the routine diagnostic work required by the field staff. Staff based at the central facility are usually responsible for compiling disease status reports, meeting the reporting requirements of regional, national and international authorities, the development of disease surveillance plans and the provision of a range of diagnostic tests and technical expertise. Although the level of development of the central and regional diagnostic facilities will vary from country to country, the general administrative and technical structure is fairly standard. The livestock owner/farmer generally does not have direct access to diagnostic services from a national or regional veterinary laboratory, the service to the farmer is usually provided through the animal health/veterinary or livestock field/ extension services.¹ Therefore, a key element in the provision and utilization of veterinary laboratory services is the link between the livestock extension officer and the farmer at the district level. This chapter will focus on the factors that need to be considered when setting up and

encouraging the use of a veterinary laboratory service.

Infrastructure and function of the regional or district veterinary laboratory

Veterinary laboratory staff have a varied and important role within the animal health services. The main contributions from laboratory staff include: (1) the development and delivery of the diagnostic service; (2) provision of technical support and training; (3) participation in disease surveillance programmes; and (4) disease reporting.

A range of veterinary professionals, technical experts, laboratory technicians, laboratory assistants and auxiliary personnel will staff veterinary diagnostic laboratories. The number and nature of staff depends on the size and functions of the facility, for example, a central or reference laboratory will have a diverse range of staff including veterinarians and discipline scientists with expertise in specific diseases or diagnostic procedures, whereas small facilities usually require all staff to have a very broad spectrum of expertise. In the larger laboratories, there may also be a research component to the work done with trainees and graduate students engaged in the work. The general requirements for veterinary laboratory facilities, including specialized facilities, are well set out in the standards adopted by the 182 Member Countries of the World Organisation for Animal Health (OIE)² and in guidelines prepared by the OIE and the Food and Agriculture Organization of the United Nations (FAO) (see also the bibliography at the end of the chapter). It is the smaller regional and district laboratories that are the focus for this book.

In regional facilities, livestock extension staff should be encouraged to liaise with laboratory staff on a regular basis, this may be via the veterinary officer in charge, or directly with the laboratory technical staff, such as when visiting the facilities to drop off samples, or when working alongside laboratory staff investigating disease outbreaks. Laboratories can provide better diagnostic services when veterinary field and extension staff are well trained so it is important that a team approach is encouraged and developed. Extension staff and field veterinarians need good training in sample selection, preservation and transportation as well as in the submission of supporting information. They will also benefit from ongoing feedback with respect to sample quality.

To maintain support for the laboratory it is important to ensure a timely turnaround of test results and an efficient response to requests for technical assistance. Regular community and professional updates from the head of laboratory facility, for example, with regard to the prevalent disease problems in an area, can also be a good way to ensure that the role of the laboratory and its staff is recognized by the wider local and professional community. Veterinary staff in the laboratory network should be encouraged to facilitate, and participate in, targeted local disease surveillance and animal health awareness programmes, in collaboration with the field and extension staff.

It is important that the laboratory service has an efficient and reliable, reporting system. This is true for reporting results out to the field veterinarians and extension staff as well as providing timely updates to the relevant regulatory authorities and reference laboratories. Regular information bulletins outlining new diagnostic procedures or disease control/treatment recommendations may also be useful to motivate field and extension staff to utilize the diagnostic services available.

Legislation and responsibility

Before planning a new laboratory facility local and national regulatory authorities should be contacted in order to check what, if any, specific restrictions and bylaws may apply to the construction of new buildings. The local regulations and provision for waste disposal, power and water supply, as well as the proximity of residential areas and commercial animal housing, must also be considered, as well as the international standards. This will be discussed in later sections of this chapter.

Education and public relations

Practical training (workshops)

The development and delivery of an effective veterinary service requires good communication and teamwork. Workshops can provide a good forum for different cadres to meet and discuss current animal health issues and through which to gain a better perspective of what each component of the animal health service can provide. Workshops can also be organized for regional and national groups of veterinary field and extension workers to promote new disease investigation campaigns and to provide a background on current diagnostic procedures. It is helpful if sample collection and sample submission guidelines are accompanied by practical demonstrations. Workshops provide the opportunity to motivate veterinary field, extension and laboratory staff to jointly

assess the performance of the laboratory and to ensure the effective delivery of services to the end user, that is, the farmer. During any planned joint activities, laboratory staff should make sure that veterinary field and extension staff have an adequate supply of laboratory submission forms and sampling materials, preservatives and transport boxes as well as providing technical advice. Specific training suggestions for laboratory technicians is outlined in section 1.3.

Field visits

Training programmes should incorporate participation in field visits, disease investigations and targeted surveillance programmes for laboratory, livestock extension and veterinary field and support staff. Joint field visits provide the opportunity for training in sample collection, as well as ensuring that good case history notes are taken, and that the quality and type of samples submitted are appropriate.

Laboratory staff should be encouraged to join field teams on a regular basis so that they can experience the practical limitations placed on livestock extension staff and field veterinarians, for example, the lack of facilities for livestock restraint, problems with sample collection and so on. Meeting the end users of the diagnostic service, that is, the farmer, can also help to highlight the importance of handling samples submitted to the laboratory with due diligence and also the need to report results in a timely manner.

The exact requirements for laboratory support and practical assistance during fieldwork will depend on the level of technical competence of the field extension staff and the availability of trained auxiliary staff to facilitate animal handling. The resources available will vary with the country and the region within the country. In urban or central laboratories, there may be little scope for laboratory staff to participate in fieldwork but in the regional and district centres there is often a strong emphasis on team effort with laboratory/extension staff taking an important role in sample collection and advisory services for the farmer.

Planning fieldwork and the use of a mobile laboratory

The emphasis of the laboratory programme will be determined by regional and national requirements. In regional centres the main role of the laboratory may be to act as a diagnostic unit whereas for many of the central facilities, research and the production of biological products will also be an important feature. Veterinary staff in regional laboratories are often responsible for providing technical advice and support as well as organizing and facilitating disease surveillance programmes, monitoring livestock at borders and quarantine units, the assessment of animal health on farms and monitoring slaughterhouse hygiene. Laboratory-based veterinarians may need to be available to provide additional technical backup for livestock extension and veterinary field staff and may be directly involved in their training.

In situations where the laboratory veterinarians, and other technical staff, are expected to provide technical backup for the field extension staff it is important that reliable and appropriate transport is made available along with an adequate budget to facilitate field visits. A robust four-wheel drive vehicle is generally suitable but may need to be upgraded if long distance journeys are required. For individual staff members, a bicycle or a motorbike may be useful for short journeys but a larger vehicle is generally preferable where equipment and sample collection materials need to be transported. Mobile laboratory units may be a good investment in some regions. These allow laboratory and extension staff to go out on tour for several days or weeks at a time and process samples en route (Figure 1.1a).

The mobile unit is especially valuable in remote areas where infrastructure is limited. The basic design for a mobile unit will depend on the work to be carried out. In most cases a refrigeration unit, work bench, suitable lighting and a wash facility for sample preparation and processing are required within the vehicle. A generator and/ or a solar panel, small incubator, microscope and disposables can be added from the laboratory store. A field microscope (that is, one with a mirror rather than requiring an electric light source) may be useful where there is no power source, with the caveat that these give a poorer resolution than a standard light microscope.

To develop a functional mobile laboratory the following questions should be considered.

- What type of laboratory equipment will be needed?
- What are the energy requirements to operate the equipment?
- How many staff will be in the team?





Figure 1.1 (a) Mobile laboratory with field team. Regular visits to rural farming communities and district extension units improves visibility and enhances public relations. (b) A district facility with mobile laboratory visiting from the regional veterinary facility. (c) Regional veterinary laboratory with small generator shed at the back of the building. (d) Regional veterinary laboratory parasitology section. (e) Regional veterinary laboratory microbiology staining sink.







- What is the expected duration of the field visits?
- How far will the vehicle need to travel each day?
- What are the road conditions likely to be?

Typical laboratory equipment for field visits might include the following:

- 18 l, 70 W cooling unit(s)
- small-size fridge/incubator: continuous use, 12 V
- LED microscope: 3 W, in use for 2 hours/day, 220 V
- microhaematocrit centrifuge: 600 W, in use for 30 min/day, 220 V.

For the above laboratory equipment, electricity could be supplied by a power inverter of around 1 kW capacity, which is typically run from a rechargeable 12 V lead acid battery or automotive electrical outlet.

A secondary benefit of using the mobile laboratory is that it raises the public profile of the laboratory facility, which encourages farmer participation in animal health initiatives.

1.2 Buildings and maintenance

The site for a veterinary laboratory must be chosen carefully as the location can determine the success or failure of the diagnostic service. The availability of effective transport and communication services, and good access are especially important. In most cases regional laboratories are built near a regional centre and district facilities are located in more rural areas were the demand for additional services justifies the investment (Figures 1.1b and 1.1c – illustrate a small district facility and a larger regional facility).

Location and design

Laboratory buildings should be located on a carefully selected site that has a well-defined compound and, preferably, land available around the perimeter to allow for future development. Owing to the potential biohazard risks the buildings should not be located near commercial animal rearing units or residential housing.

The design of laboratory buildings generally follows a standard plan. An example of a design submitted for a regional veterinary laboratory is illustrated in Figure 1.2. Plans for district laboratories will depend on the specific needs of the area and the budget available. In many cases district laboratories are attached to district livestock extension or veterinary centres. This facilitates sharing of resources and can enhance communication between different cadres of the animal health service. The details of the design for district and regional laboratories tend to depend on local building regulations. Reliable plumbing and power connections are especially important. The style of the out-buildings and the interior fixtures will vary depending on the budget and the building materials available locally but piping and drainage should be of the highest quality. Short-term cost savings often result in significant maintenance problems in the longer term so the quality of construction should be suitable and supervised by a site manager familiar with acceptable laboratory construction standards. For example, the flooring should be smooth and have a continuous join with the walls to facilitate disinfection and cleaning. Ventilation and waste disposal should be well planned. In laboratories that deal with zoonotic diseases and high-risk pathogens there needs to be a designated section with suitable isolation and bio-containment facilities.³ Ensuring that there is effective waste disposal for biological and chemical wastes from laboratories is essential. This should be efficient and environmentally safe. In most countries, there will be local environmental and public health regulations that will need to be taken into consideration before the laboratory is built. Most laboratories will require a sump tank and septic tank for waste water and sewage to avoid contamination of local water supplies and waterways. Some biohazardous waste may be taken away by municipal authorities but this service is often not available in remote areas. Toxic chemicals should be stored in containment facilities until suitable bulk disposal can be identified. Carcasses and biological wastes that cannot be incinerated will need to be disposed of in a specially built biological pit as illustrated in Figure 1.4.

Services

The laboratory will not function effectively if the relevant services are not available for the routine day-to-day work in the facility, that is, reliable water and power supply. Transport and communication will be considered later.

Power supply: electricity and gas

A reliable power supply is essential for the efficient running of a laboratory.

ELECTRICITY

Electricity is generally provided by the following sources:

- municipal power
- fuel generator
- dry alkaline batteries
- · solar energy panels.

In most central veterinary diagnostic laboratories, the power supply is likely to be from a municipal electricity grid system. This source may not be available or reliable in remote rural areas.

When the quality of the power source is irregular (power surges, voltage drops), sensitive equipment such as computers, microscopes and freezers should be linked up to a power



Figure 1.2 Simplified ground plan for a regional laboratory. Note that the flow of specimens in the laboratory should generally be split between the 'clean' and 'dirty' areas. The latter includes the post-mortem and parasitology sections. The size and design for a district laboratory will depend on work requirements, budget and animal health priorities identified in an area.

stabilizer. Apparatus that cannot withstand power cuts (polymerase chain reaction [PCR] cyclers, spectrophotometers, fluorescence microscope and so on) should be connected to an uninterruptible power supply (UPS) or to a 'no-break' battery power supply system. Preferably a central UPS or 'no-break' system, which can automatically switch between the mains power supply and battery power with continuous charging when mains power is available. The capacity of the UPS/'no-break' and power stabilizers should be selected to match the requirements of the apparatus that could be damaged by fluctuating power. Instead of using a stabilizer for dedicated equipment is usually preferable to install a more powerful stabilizer for the whole laboratory, for example, via a generator.

There are a number of suitable generator units available commercially (typically in the range of 8 to 12 kVA), which are generally reliable and economical to run. In most cases, unless the laboratory is very small, the generator will be set up to supply only essential pieces of equipment during a power failure so it is important to identify and mark the outlet sockets that will be supplied with power when the generator is operating.

Where the electricity supply is unreliable, consideration can be given to purchasing equipment that does not require electricity. Some refrigerators and autoclaves, for example, use a gas or kerosene power supply.

For ensuring the provision of power to maintain cold facilities it may also be possible to use solid carbon dioxide or liquid nitrogen, if stocks of these are readily available and are not too costly.

Solar energy supply systems can also be used in remote laboratories and in areas where the electricity supply is erratic. Solar powered refrigerator/freezers are already widely used. Maintenance of solar panels (that is, cleaning and dusting regularly) and the batteries is important to extend the life span of the system. Some equipment can be run on alkaline batteries or rechargeable internal batteries such as the EKF Diagnostics Tm haemoglobin meter.⁴ Microscopes with LED-illumination equipped with small solar panels can be used for several hours without mains supply. Other technologies are being developed to help facilitate work in areas where power supplies are unreliable, because of this, it is good to keep up to date with scientific developments via local equipment suppliers and professional organizations.

GAS

In some areas gas may be supplied via a public mains system or alternatively it may be supplied in liquid form in storage cylinders that can usually be obtained through commercial suppliers. Individual gas cylinders can also be placed under the benches in some laboratory sections (for example, microbiology) so that they can be connected to single outlet points. This is especially useful for small laboratory units where piped gas is not feasible. However, storage of such cylinders is subject to local safety regulations. Spare cylinders should be kept in a well-ventilated storage room in a building separated from the laboratory and suitable fire precautions should be in place.

A laboratory may have a set of large gas storage cylinders maintained outside the building. These should have suitable protection from weather and safety measures in place in case of fire.

Water supply

A reliable supply of clean water is essential for a laboratory to operate. Water is used for all aspects of laboratory work from sample preparation to test procedures, washing and general disinfection. The water quality may vary considerably so the addition of appropriate filtration and purification systems is required to keep the supply suitable for laboratory requirements (see Chapter 2). If there is no reliable piped public water supply it is advisable to install storage tanks with as large a capacity as possible. These can be fixed to the roof to facilitate water flow, however, a well-fitted pump system is better than utilizing gravity flow alone. The water pump should be linked up with a power stabilizer or a power trip switch-voltage protection unit to prevent damage caused by a fluctuating power supply.

In district laboratories that have an intermittent water and electricity supply, it is essential to have a backup to ensure that water is available each day to allow the laboratory to function. For example, an underground storage tank built outside the laboratory that can be replenished either by rainwater (run off from the roof), or in the dry season, with water brought by bowser from a central supply. Water can then be hand pumped into an overhead tank and supplied by gravity to the lab. If a storage tank is built it is important to add suitable filters to prevent the build-up of silt, which can soon cause a blockage in the pipes.

Hot water can be supplied from electric- or gas-heated geysers; this is especially important for the wash room. To reduce the use of hot water from boilers, try to install the distillation apparatus in the washing room. This way, the cooling water from the distillation apparatus (temperature about 60°C) can be used for cleaning laboratory glassware and other items that need washing.

Distillation and de-ionization of water is outlined in more detail in Chapter 2.

Transport and communication services

The availability of reliable local transport and communication services is very important for the functioning of the laboratory and also for the well-being of laboratory staff. If a laboratory is built in a place remote from normal public services it may be difficult to recruit and retain staff. Lack of good transportation and communication networks can also make it difficult to encourage sample submission.

Managing supplies

The selection, storage and distribution of supplies is an important aspect of laboratory maintenance and service provision (see Chapter 2). When purchasing equipment, appropriate technology should be selected depending on practical considerations as well as the needs of the unit. Careful thought must be given to maintenance costs as well as the longerterm requirements of a laboratory unit. This is especially true with regard to reagents and disposables, for example. Kit tests may be preferable to more laborious methods in the short term but if the cost of replacement reagents or kits is too high then such tests may not be sustainable.

To ensure a regular flow of general supplies and spare parts for equipment it is important to set up an efficient store system. If stores are not well managed the day-to-day work of the diagnostic unit may be impaired especially where deliveries take several days (or weeks) to arrive or where the funds for new purchases are unavailable. Careful stock control and good forecasting of the requirements for each laboratory section at the beginning of each financial year are essential.

Stock control

The functioning and maintenance of a good store room requires a well-organized stock control and ordering system. The amount of stock held in a central or regional store will depend on the availability of funds, and the projected needs of the laboratory and the associated animal health network. The ease of ordering will also determine how often purchases can be made. Planning ahead is important but until a laboratory has been in operation for a few years it may be difficult to accurately assess the requirements ahead of time.

Local distributors and dealers for hardware and equipment can usually provide guidance on, or facilitate the procurement of, associated reagents and disposables. However, in most developing countries it can take some time to procure laboratory supplies. There are several reasons for this.

- 1 Supplies are often not available in the country and there are no reliable agents.
- 2 Procurement procedures are cumbersome.
- 3 Delivery and transport times are long.
- 4 Foreign currency may be required for online purchases, but is unavailable.

Difficulties in getting adequate supplies of equipment, consumables and reagents can account for major disruptions to work programmes. The time between a request for supplies and receipt of the order in regional and district laboratories can often be several months. This will depend, to some extent, on whether the laboratories have their own budget and stock control system or whether every order needs to be directed through a central laboratory. In most cases the regional and district facilities will have a small budget for consumables but larger expensive items are purchased centrally. However, this varies from country to country due to administrative variation and logistics.

Where there are a lot of remote district laboratory units it may be preferable to place all orders for disposables, and general reagents, through a regional or central facility. Buying in bulk is often cheaper and stock control can be better maintained. If specific disease surveillance projects are planned, the requirements for consumables should be outlined at the beginning and a specific budget identified to support the planned work. Stock control can be computerized but in the smaller centres it is often still based on manual records. Both systems require frequent checking, updating and forward planning. To ensure consistency a designated staff member should be given the responsibility of maintaining up-to-date stock records.

Store sections

The store room(s) will need to be set up in a tidy and well-organized manner so that the levels of stock can easily be checked by eye as well as by checking the stock books or the computer database. In most cases chemicals will be stored in a separate room from the consumables and equipment. Dangerous or flammable chemicals will need to be stored in a separate, concrete lined, room. All stock rooms should be free of vermin and readily cleaned. Access should usually be limited to one or two store keepers and the laboratory supervisor. Up-to-date records of in-coming and out-going deliveries should be kept and delays in either the arrival or dispatch of orders should be followed up as early as possible.

A store list will usually be organized into sections. The following is a suggestion of the categories to consider:

- 1 glassware
- 2 plastic ware
- 3 laboratory ware
- 4 media
- 5 stains and poisonous chemicals
- 6 acids and other corrosives
- 7 alcohols and other flammable materials (these should be kept stored in purposebuilt fire-proof rooms)
- 8 instruments and equipment
- 9 spare parts for equipment
- 10 perishable reagents (including refrigerator).

A note on isolated district laboratories

District or 'satellite' laboratories are often established in isolated rural areas as a focus point from which to provide a basic diagnostic service and technical advice to the rural livestock extension network. This is especially common where the transportation between regions is slow or services limited. The district facilities are usually supplied with basic equipment, test reagents and consumables through the nearest regional laboratory. District animal health extension staff may also have an office located close by the laboratory, where they will have basic medicines and vaccines in stock. The tests performed in these smaller facilities are usually restricted to basic parasitology procedures and simple microbiological tests, such as screening milk samples for mastitis. The staff responsible for the district facility will often take samples to a regional laboratory if additional laboratory tests are required. District facilities can improve direct services to the farmer through collaborative efforts between laboratory and extension staff. However, it is important to ensure that isolated laboratory units are given sufficient logistical and technical backup from regional and central units. Staff morale can deteriorate rapidly if resources are too restricted and regular contact is not maintained.

1.3 Staff requirements

Fieldwork

Field programmes based at small district laboratories can be run in conjunction with other livestock extension projects (such as artificial insemination schemes, vaccination or educational programmes). This can save time and resources and also encourages teamwork. The number and cadre of staff required for fieldwork will depend on the nature and the size of the project. At the district level, it would be common for all members of the team to share in routine work, such as preparing sample collection equipment, collection of samples and initial preparation of specimens for field based laboratory tests. It should be noted, however, that there may be specific regulatory requirements outlining job descriptions for staff, and government or provincial legislation outlining what technical staff may do in the field, which should be checked for the country or area in which the work will be conducted.

Laboratory

The quality of a laboratory is directly dependent on the training and performance of the laboratory personnel. The laboratory management in charge of the laboratory is generally responsible for the following.

- Deciding the grade (laboratory assistants, technicians, senior technicians, veterinary scientists/specialists, team supervisors and so on) and number of laboratory personnel required to staff the service.
- Preparing job descriptions for each grade of laboratory worker and determining the qualifications required for each grade.
- Employing suitably qualified personnel and provision of training and career development.
- Developing standard operating procedures (SOPs).
- Ensuring that health and safety regulations are complied with.
- Ensuring that quality standards are maintained (for example, for laboratory accreditation).

The requirement for laboratory staff depends on the size of the laboratory unit and the anticipated workload, as well as on the degree of technical competency required. A small regional laboratory may function efficiently with a team of two or three technicians and a senior technical supervisor or a veterinary officer, plus two or three auxiliary staff. Some of the administrative aspects of the laboratory may need to be handled by qualified veterinary officers, especially where diagnostic advice and decisions on disease control and prevention procedures need to be made. The legal requirements will vary from country to country and will, to some extent, dictate the staffing policy.

In larger central and regional laboratories, there may be a senior veterinary officer and several other veterinary professionals working in an aligned disease surveillance unit as well as those placed within each diagnostic discipline in the laboratory. Where laboratory training has been emphasized there may also be senior laboratory technicians and research staff who take a lot of responsibility for the running of specialist laboratory sections and who may supervise teams of discipline specific technicians. The staffing levels will be determined by the needs of the animal health services, the budget available for the service and the availability of competent trained professionals.

Training laboratory technicians

Curricula for training laboratory technicians will often depend on the scope and level of laboratory in which they work, that is, district, regional or central level, and will vary according to the animal health needs prevalent in the region. Core competencies recommended for entry-level veterinary laboratory technicians are outlined in the OIE Competency Guidelines for Veterinary Paraprofessionals published in 2018.⁵

Basic training, in both the theory and practical aspects of laboratory testing is required for newly appointed laboratory technicians. In some cases, laboratory technicians entering the veterinary sector may have already had good foundational training through available biomedical laboratory training programmes. While these biomedical laboratory technicians may be highly competent to perform some of the core functions in a veterinary laboratory, they will need some additional training to become fully competent in the veterinary diagnostic service.

In some countries, there are already welldeveloped training programmes established for veterinary laboratory technicians. These generally offer well accepted qualifications at the certificate, diploma or degree level. Formal qualifications may be obtained over one or several years or may be gained later in conjunction with 'on-the-job' training. However, in some cases tailored, short courses may be needed to provide enhanced technical capacity for new animal health programmes requiring more veterinary laboratory support.

For small district laboratories, basic entrancelevel training might be achieved in a short intensive course (see below) followed by supervised 'on-the-job' training. In many cases the theoretical aspects of the course material can be delivered in modular form and laboratory staff can build their qualifications from the certificate level through to a more advanced level over time.

Key topics for short intensive courses could include the following:

- laboratory biosafety and biosecurity
- · quality management
- laboratory techniques (practical and theory)
 - microbiology
 - parasitology
 - clinical chemistry
 - pathology
 - haematology
 - serology
- · professional ethics
- communication skills
- applied anatomy and physiology
- animal diseases common in the region
- field sampling.

Curricula for trainees in larger laboratories, where a larger range of tests might be performed, would need to be more comprehensive and may take up to 1 year or more to complete. The formal requirements for obtaining specific qualifications in laboratory technology will vary from country to country depending on what is supported by 'in country' academic institutions and the relevant regulatory authorities. In most cases, it is preferable to select trainees that already have a good high-school education in biological sciences, applied mathematics, physics and chemistry. Entrance-level qualifications for laboratory assistants and support staff will vary depending on the scope of the job.

It should be noted that in some situations there might also be highly competent laboratory technicians without formal qualifications but who have gained years of valuable experience on the job.

At the end of a formal training programme, laboratory technicians should be assessed on both their theoretical knowledge and practical skills. Newly trained laboratory technicians will need ongoing supervision and support once they reach their province/district. This should be done by a designated laboratory trainer or supervisor. Continuous training in the workplace is one of the most effective ways of maintaining and upgrading the knowledge and technical skills of laboratory staff.

To ensure the quality of the veterinary laboratory service it is recommended that there are regular visits (by experienced staff from the central laboratories to provide ongoing mentorship and technical training support) scheduled to district facilities to assess the performance of personnel. In addition, regular (at least once a year) refresher training opportunities should be available through the larger regional or central veterinary laboratory. These courses provide a good opportunity to introduce and explain the use of new techniques and technologies. Training in basic laboratory techniques can also be provided to livestock extension and veterinary staff so that they have a better appreciation of tests done in the laboratory. Specific training can also be provided in the use and interpretation of results from common field tests (for example, mastitis screening tests, slide agglutination tests, pen side diagnostic kits and so on).

The importance of ongoing educational support and mentorship for all staff in the animal health services cannot be overemphasized.

1.4 Safety in the laboratory

Access to the core part of the diagnostic laboratory should be restricted to authorized employees. Clear signage should be in place to ensure that unauthorized visitors do not enter beyond the sample submission or registration area. Every member of staff working in a veterinary laboratory, including administrative staff, should be made aware of the possible risks associated with handling potentially hazardous biological material.

Some animal diseases, for example, rabies, hydatids, bovine tuberculosis, brucellosis and salmonellosis are zoonoses, and material from suspected cases of these and other diseases must be handled very carefully. Staff should have knowledge of common zoonotic diseases (see Appendix A1) and if the risk of exposure to specific diseases of concern (for example, rabies, tuberculosis and so on) is high, vaccinations (if available) are recommended. Guidelines for personal protection are provided by the World Health Organization (WHO) but each laboratory should also develop a policy and associated operational guidelines to ensure the health and safety of staff with specific attention to the diseases and hazards common to the region and/or facility. The OIE provides standards for biosafety and biosecurity for veterinary laboratories and

animal facilities in Chapter 1.1.4 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. A few general considerations are provided below.

In general, any laboratory sample from a dead or sick animal should be considered potentially hazardous to humans. Precautions should be taken to prevent contamination of the laboratory benches and equipment as well as making sure that strict procedures for personal hygiene are observed. It is generally recommended that disposable gloves are worn when handling specimens. It is recommended practice to undertake risk assessments for handling different types of samples, or for undertaking specific activities, and these are then used to generate SOPs. The microbiology section of the laboratory should always be considered a restricted area and only the staff directly involved in microbiology work permitted to enter. When centrifuging samples of potentially hazardous material, the sample container should be securely sealed before being placed in the centrifuge. There should be protective clothing (laboratory coats, masks, safety glasses, gloves and so on) and biosafety cabinets available for staff handling hazardous specimens, that is, from cases where a zoonotic disease is suspected (for example, tuberculosis, psittacosis and other biological agents). These points are discussed further below.

Handling potentially infectious material

The senior laboratory supervisor will often contact a consulting medical practitioner or public health officer for advice on disease prevention protocols and for the selection of personal protective equipment. Basic guidelines as well as pathogen specific recommendations are provided by the WHO⁶ although each laboratory should develop its own guidelines based on a locally relevant risk assessment for both infectious and non-infectious hazards in the laboratory. Identified risks can be mitigated using SOP and good laboratory practice but staff will also need to be trained. This training should be updated when new risks are identified. Some general principles are outlined below.

- 1 All specimens received in the laboratory should be regarded as potentially hazardous and handled with due care.
- 2 Prevention of exposure to potentially infectious agents is very important. This requires training staff in both good laboratory practice as well as making them aware of common zoonotic diseases and how to prevent disease transmission.
- 3 Provision of appropriate protective gear, including masks and respirators for handling highly infectious pathogens, is essential in laboratories where samples may contain organisms that may be transmitted by aerosol. Such samples can also be handled in a biosafety cabinet.
- 4 Protective clothing should be worn at work, and removed when leaving the designated work area. This can include coveralls or plain white laboratory coats and disposable gloves, sturdy footwear and so on.
- 5 Special protective clothing (that is, masks, protective goggles, rubber boots, washable or disposable aprons and so on) should be worn when working with material from cases of suspected rabies or other zoonotic diseases, and at post-mortems. In countries where rabies is common, all staff should be vaccinated and the post vaccination titres checked to ensure protection.
- 6 Always ensure that staff wash their hands thoroughly with soap and water after handling cultures and/or specimens and before leaving the laboratory.
- 7 Do not smoke or eat in the laboratory.
- 8 Spillage of potentially infectious material should be handled as follows:

- i disinfect immediately (wear gloves!)
- ii cover with disposable tissue (to make the spill visible)
- iii warn colleagues that there is a spill
- iv keep the spill remains covered with the disinfectant for about 30 min
- v wipe up the spill using absorbent paper and discard in the biological waste bin.
- 9 Soiled swabs, microbiology samples, cultures and all potentially pathogenic material should be discarded in the biologic waste bin (not in the waste paper basket).
- 10 All glassware and containers used for potentially pathogenic material must be placed in a disinfectant before sterilization and washing.
- 11 Benches should be wiped down every day with disinfectant in the morning and before leaving the laboratory.
- 12 Used, contaminated sharps (needles, Pasteur pipettes and so on) should be discarded in a safe 'sharps container'. In many countries, there are contractors who will provide waste disposal for veterinary and medical facilities, they usually supply sharps containers and other receptacles which are collected for disposal. These contractors must abide by local and national byelaws.

In addition to biological hazards, there are many potentially dangerous chemicals stored and used in the laboratory, such as strong alkalis and acids, which can cause burns and damage eyes. Many reagents, and their vapours, such as alcohol and ether are flammable and only small amounts should be kept in the laboratory in order to reduce the associated fire hazard.

A designated member of staff should be identified and trained to take responsibility for first aid. However, all staff should know which chemicals are hazardous and what measures should be taken to prevent accidents. Every laboratory should also have a first aid box containing emollient creams, an eye bath and an assortment of bandages and plasters. Many laboratory facilities also provide an emergency shower in case of skin contamination. The regulatory requirements for occupational health and safety will vary from country to country and also depend on the designated biosafety level for each division of the laboratory. Most diagnostic laboratories will have sections with level 1 and level 2 biosafety designations. Those with level 3 and higher are highly specialized and will have very clear health and safety guidelines for handling specific pathogens. WHO provides a good summary of what is expected with respect to health and safety training and best practice (see also the bibliography at the end of the chapter). In most countries, the national authorities responsible for public health will also have specific guidelines that must be adhered to.

As outlined earlier, potentially hazardous biological wastes and carcasses are usually disposed of in a specially constructed pit in which biodegradation can occur (Figure 1.4). The pit should have a sealed lockable lid and a lime-sealed concrete surface, which can be washed down after performing post-mortems. No disinfectant or non-biodegradable material should be put in this pit as this will delay decomposition.

Potentially contaminated material from microbiology and parasitology sections should be soaked in disinfectant (for example, phenolics) before being disposed of (not in the biological pit). Contaminated equipment, glassware and consumable materials for re-use (for example, Petri dishes, microscope slides) should be disinfected before being thoroughly washed in detergent and several changes of distilled water. Many laboratory chemicals and biological wastes are hazardous to the environment and should be disposed of carefully to prevent pollution of local water supplies.

Legislation (national and local authorities)

Legislative requirements with regard to health and safety procedures, as well as biosecurity requirements, vary from country to country. It is important to contact local authorities *before* a laboratory is built to determine how any local regulations may affect the day-to-day functioning of the facility. Special rules may apply to the training of staff and the use of safety equipment, provision of containment facilities and so on.

Waste disposal and biosecurity

Laboratory staff have the responsibility to protect themselves, customers, the community and the environment from injury or damage originating from infectious or toxic laboratory waste and to minimize the hazards involved in decontamination, recycling and disposal. These risks can be minimized by laboratory staff knowing and following correct methods for:

- separating infectious materials and laboratory waste
- decontaminating and disposing of non-reusable laboratory consumables and infectious waste
- cleaning and sterilizing reusable consumables and equipment.

It is the responsibility of the laboratory supervisor to avoid environmental pollution and to minimize the potential danger to the staff within the facility and the general public.

The waste fractions in Table 1.1 are gathered separately by the people working in the laboratory. Some of these fractions should be treated before disposal.

Non-biological, non-combustible wastes (that is, broken glass, decontaminated sharps, some plastics and metals) can be stored in a well-constructed waste store (that is, with solid concrete

Table 1.1 Handling of common laboratory wastes.

| Waste fractions | Treatment | Disposal |
|---|-------------------------------|--|
| Infectious waste (used bacteriological media, used swabs, etc.) | Steam sterilization | Remainder waste (incinerator or approved dumping ground) |
| Carcasses | Biological pit or incinerator | lf incinerator is used: ash (remainder waste – dumping ground) |
| Used sharps (infected or not) stored in sharps containers | Steam sterilization | Stored in plastic containers in a waste store until a professional waste treating company can take care of it. Never bury sharps |
| Dirty reusable not infected ware (glassware, plastic ware) | Washing | The water used to clean the laboratory ware should be removed through a separate sewage system if possible |
| Dirty non-reusable not infected items | | Remainder waste (incinerator or approved dumping ground) |
| Paper, non-infectious plastic | | Remainder waste (incinerator or dumping ground) |
| Dangerous chemicals | | Should be disposed of according to local authority regulations through professional waste disposal services (if available). |

walls). Dangerous chemicals and some plastic materials should be disposed of according to local authority regulations through professional waste disposal services (if available). Chemicals should never be buried because they may leach out into the water table and poison the water and soil. Avoid burying or burning sharp objects, such as needles, as these can be picked up by the public or their animals and can cause injury.

Combustible waste (that is, paper, cardboard) can be burned. A suitable container for burning such waste can be made from an old oil drum by making holes in the sides and bottom and removing the top (Figure 1.3). The drum should be set on a foundation of concrete, bricks or stones to allow airflow underneath and it should be held firm to prevent it from falling over. It is important to construct a secure fence around the waste disposal area to reduce the risk of interference from local children or animals.



(use concrete bricks or stone)

Figure 1.3 An old oil drum or a metal rubbish bin can be used as an incinerator. (A) Old metal drum or bin with holes made (B) to allow air flow, this improves combustion. (C) Stone or concrete platform to elevate the base of the incinerator from the ground. Note that there must be holes at the bottom of the drum to ensure air flow so do not use a solid platform. Illustration: Louis Wood.

Incinerators

Incinerators can be an appropriate investment for some laboratories as a means of disposing of biological waste material. Large industrial incinerators can be used to dispose of carcasses and other biological material but are very expensive to set up and maintain. In addition, spare parts may be expensive or hard to obtain. A simple low-cost incinerator for combustible waste or non-hazardous material is illustrated in Figure 1.3. This type of incinerator is often used to deal with low-volume waste materials generated by small laboratory units.

Biological pits

A simple, inexpensive and efficient method of dealing with biological waste (including carcasses) is to construct a biological pit. A biological pit may be constructed to back up an incinerator in case of servicing or repair, or as the main means of disposing of biological material, which includes carcasses of large and small animals and fresh, unfixed necropsy specimens.

The pit should be situated and built with due regard to any underground fresh water sources such as wells and springs. The proximity of rivers and other natural water sources should also be considered. To avoid the odour of decomposing materials the pit should be a minimum of 5 m deep. A plan for a pit is given in Figure 1.4 and this can be adjusted to suit local conditions. The dimensions of the pit will depend on the expected amount of material to be disposed of. The surface of the pit will need a well-constructed impervious cover on which post-mortems can be conducted if necessary and to allow regular surface washing and easy disposal of animal waste into the pit. The lid should be strong and preferably have a lock to prevent unauthorized access. Disinfectants, preservatives or any antibacterial chemical should not be used on the surface of the pit cover nor should



Figure 1.4 Diagram of a biological pit (longitudinal section). The diameter and depth of the pit will depend on the volume of material expected to be put in it. Most are circular in cross section with a diameter of 2–3 m and a depth of 4–6 m. Only biological materials should be added and no chemicals such as disinfectants or antibacterials as these will delay (or even prevent) biological breakdown. (A) Brick, earth or stone lining to a depth of 2 m. (B) Concrete apron (easy to keep clean), surface edge sloped for drainage (X). (C) Metal lid. (D) Fly trap (plastic tube with clear elevated plastic top). (E) Earth. (F) Open end to allow natural drainage.

such materials be washed into the pit as this will interfere with decomposition.

Vermin and flies should be controlled to avoid the risk of spreading disease. For this purpose, most pits are built with a fly trap inserted near the lid. A well-kept biological pit relies on bacterial breakdown of organic matter and can provide an efficient and safe way of disposing of biological wastes. To assist natural decomposition, various commercial mixtures of bacterial culture can be added, that is, those used in some domestic septic tanks. Earthworms may also be used to provide aeration. The pit must have a strong, preferably metallic and lockable lid.

Procedures and protocols

As outlined earlier, legal requirements may vary from country to country and should be reviewed and obeyed with regard to health and safety regulations. The following are examples of rules that could be posted within the laboratory:

Apparatus

All equipment is potentially dangerous if it is faulty or not operated according to the manual/ operating instructions. Any faulty or damaged piece of apparatus should be immediately reported to the chief technician or laboratory manager and not used before it is repaired.

Glassware

Glassware with damaged edges should not be used because it is dangerous and may be inaccurate. Damaged glassware should be discarded into designated receptacles and not in waste paper baskets. Working space should be kept clear of unnecessary glassware.

Pipettes

Pipetting by mouth is not acceptable. Always use a pipette teat or a mechanical unit for pipetting acids, alkali, poisons and samples of potentially infectious material.

Knives and sharp implements

Knives and other sharp or pointed instruments should be cleaned and put away carefully in a designated box immediately after use. Do not leave sharp instruments on the bench or loose in a drawer.

Chemicals and reagents

- 1 All bottles and containers should be labelled clearly to show their content and date of preparation. Observe warnings on containers and act accordingly.
- 2 Know the harmful effects and potential danger of chemicals used in the laboratory and how to store them correctly.
- 3 After using strong acids or alkalis be sure to wipe the neck of the bottle before returning it to the shelf.
- 4 Neutralize and wipe up immediately any acid or alkali that is spilled.
- 5 Take extra precautions when working with chemicals which produce a toxic or irritant vapour (that is, only use in a biosafety cabinet, wear protective glasses, masks, and gloves and so on).

Fire prevention and control

When working with highly flammable chemicals the danger of fire should always be kept in mind and adequate precautions taken. Flammable chemicals include ether, benzene, xylene, toluene, acetone and alcohol. It must be remembered that there is a fire hazard from the fumes given off from some chemicals, for example, ether, so all combustible chemicals must be securely stoppered when not in use. All members of staff should be familiar with the location and use of the fire apparatus adjacent to their laboratory. No one should smoke in a laboratory.

1.5 Clinical examination, sample selection, submission and clinical diagnosis

Clinical examination

A clinical examination of any animal should be thorough and systematic. It is important to follow the same procedure every time to ensure that as much information as possible is obtained from each case. In order to determine whether or not there is an abnormality it is necessary to be familiar with what is normal for the species of animal to be examined. The normal range for body temperature, heart rate and respiratory rate for common domestic species are provided in Table 1.2.

A clinical examination should be made with the animal at rest. Temperature (T), heart rate (HR) and respiration rate (RR) should be evaluated early in the course of clinical examination. The HR, RR and T may rise with excitement or fear and will also be elevated after exercise.

Before a clinical examination can take place, it is important that the animal is safely restrained. Some aspects of clinical examination, sample collection and restraint are illustrated in Figures 1.5 to 1.31. Care must be taken that neither the animal nor the handlers are injured. For this purpose, it is important to be familiar with animal handling techniques and always carry extra restraint ropes. If it is known that the animal(s) will be difficult to handle make sure that there is extra trained help and veterinary assistance to

| Species | Dog | Sheep | Horse | Cow | Pig |
|----------------------|--------------|--------------|--------------|--------------|--------------|
| Temperature °C | 38.9 +/- 0.5 | 39.1 +/- 0.5 | 37.6 +/- 0.5 | 38.5 +/- 0.5 | 39.5 +/- 0.5 |
| Temperature °F | 102 +/- 1 | 102.3 +/- 1 | 100 +/- 1 | 101 +/- 1 | 102.5 +/- 1 |
| Heart rate/min | 100–130 | 75 | 44 | 60–70 | 55–86 |
| Respiratory rate/min | 22 | 19 | 12 | 30 | 20 +/- 5 |

Table 1.2 Normal clinical parameters for various domestic animals.



Figure 1.5 Restraining an indigenous cow for examination and blood sample collection, Khaling, Eastern Bhutan.



Figure 1.6 Collecting samples for the diagnosis of foot and mouth disease using a sterile swab in a foot and mouth disease endemic area.





Figure 1.7 Field team restraining a young cow for sample collection, Khaling, Eastern Bhutan.

Figure 1.8 Restraint of a sheep for examination and trimming of the feet.



Figure 1.9 Samples which can be collected from a live animal. For more information see the relevant sections in Chapters 3–8.

provide chemical restraint if required. It is the responsibility of all veterinary and animal health staff to make sure that neither they nor the people assisting are injured. When part of a disease investigation it is important to make sure that a good health history of the farm or village livestock is taken. The following guidelines are general, more detailed information about specific problems is provided in other chapters (see index). The samples which may be selected for specific clinical presentations are outlined in Chapter 9 and in the relevant chapters on specific disciplines.



Figure 1.10 Restraining a cow by means of a bull holder. Illustration: Louis Wood.



Figure 1.11 Restraining a cow by grasping the nasal septum and one horn. Illustration: Louis Wood.



Figure 1.12 Ropes can be used for casting cattle for examination especially where there are no suitable handling facilities. If animal handlers pull slowly on the head ropes (B) and the body ropes (A) the animal will fall to the far side. Attend a training course to learn the correct way of applying the ropes.



Figure 1.13 Restraining a sheep by turning it on its rump. Blood samples can readily be collected from the jugular vein in the neck (see also Figure 1.18).



Figure 1.14 Application of a twitch (a) to control a mule (b and c). Most equids will accept the twitch but do not twist the pole too hard as this will damage the upper lip. Illustration: Louis Wood.



Figure 1.15 The initial examination of a 'downer' cow may be straightforward if the animal is resting comfortably but to determine the cause of the problem it is important to obtain a full clinical history (that is, has the cow calved recently? Was it a difficult calving? Is the cow a high producing animal likely to develop hypocalcaemia and so on). Temperature, heart rate and respiratory rate should be noted as well as general condition, presence or absence of normal abdominal sounds, evidence of trauma, colour of mucous membranes, consistency of faecal material, colour of urine, and so on. Blood samples may need to be collected to assess the severity of the animal's condition.



Figure 1.16 Mouth gag for cattle (drinkwater gag), which is placed on one side of the jaw to hold the mouth open. If a bovine animal has difficulty eating, often drops food, or appears to be salivating a lot it is important to look in the mouth. In some cases, it is necessary to use a gag and a torch to allow good visualization of the throat. If rabies is suspected seek advice from the regional veterinary officer *before* examining the animal. Illustration: Louis Wood.



Figure 1.17 Using the ball of the finger (not the thumb) the pulse can be measured at the middle coccygeal (tail) artery in the bovine. Blood samples can be collected from the middle coccygeal vein. Illustration: Louis Wood.





Figure 1.18a–d (a) The anatomy of the neck of the ruminant (topographical features). This illustrates the location of the external jugular vein which is a common site for the collection of blood samples. The paired jugular veins extend the length of the neck and terminate within the thoracic inlet. The jugular veins return blood from the head to the heart via the cranial vena cavae. (b, c and d) These photographs demonstrate blood collection from the jugular vein of a dairy cow using a wide bore needle and vacutainer. Photo: Dr Regula Waeckerlin, Faculty of Veterinary Medicine, University of Calgary.



Figure 1.19 Examination of the udder of a cow. Note the presence of any swelling, unusual heat or redness or evidence of pain during palpation. Examine each teat and the milk secreted from each quarter. If the milk is discoloured or very thick it may indicate that the cow has mastitis but it could also mean that the cow has recently calved so make sure that the animal is examined carefully and check the case history. Illustration: Louis Wood.



Figure 1.20 A simple test for mastitis is the California Mastitis Test which is available as a kit. Illustrated is a representative plastic squeeze bottle (A) which contains the reagent for the test and a typical plastic paddle (B). The paddle has four shallow cups which can be marked to indicate which guarter of the cow's udder the milk sample was collected from. A few drops of reagent are added to 6-7 drops of milk from each quarter. If the milk sample precipitates it indicates that there are inflammatory proteins and cells present. Often only one quarter of the cow's udder may be affected. If it is necessary to collect a milk sample for microbiological culture collect it aseptically. To do this, wipe the teat(s) with an antiseptic before squeezing the teat to collect the milk. Usually the first few drops are discarded and 3-5 ml collected into a sterile jar. The jar should be labelled to indicate the date, the identity of the animal (age, breed, tag number and so on) the guarter of the udder affected, the name of the farmer and the submitting animal health professional. In most cases the veterinary officer would add a case history to indicate the presence or absence of other clinical signs and the health history of the animal. Illustration: Louis Wood.



Figure 1.21 Examination of breeding and neonatal animals. If a farmer suspects that there are health problems in breeding or neonatal animals it is important to get a good history for the entire group of animals to allow an assessment of the extent of the problem. There are often a number of factors which cause poor neonatal survival and/or abortion and infertility. Epidemiological information (that is, looking at the pattern of the disease) is often more useful than collection of laboratory samples unless specific causes can be investigated. To examine individual cases, follow the normal routine of clinical examination and collection of a case history. There are a range of good text books available which outline the main diseases affecting various age groups of livestock, these are referred to at the end of the chapter.



Figure 1.22 Examination of a mule. The pulse can be taken at the submandibular artery. Illustration: Louis Wood.

Figure 1.23 If it is necessary to look into the mouth of equine species or to rasp the teeth, a gag such as the Haussman–Dunn gag (illustrated) may be required. Illustration: Louis Wood.



Figure 1.24 Illustration of the area of the chest to listen to in the horse (a) or the cow (b) if it is necessary to assess changes in respiratory sounds. The location of the heart and rib cage in the bovine is indicated in (b). It is important to be familiar with the normal anatomy and physiology of common species examined. Illustration: Louis Wood.

Figure 1.25 Lifting a forelimb of a horse may allow examination of the limb/foot and will restrain the animal if it will not keep still. Ropes should be held firmly. In most cases, it is preferable to get an experienced horse handler to assist. Only try to collect blood samples (usually from the jugular vein) if the animal is appropriately restrained or sedated. Illustration: Louis Wood.





Figure 1.26 Using a small board to assist in moving a pig to a suitable area for sample collection. Illustration: Louis Wood.

Figure 1.27 Collection of a blood sample from the ear vein of a pig using a 22 gauge needle and a syringe. For some samples, it may be preferable to use a vacutainer (this is discussed further in Chapter 5). A twitch has been applied to the upper jaw of the pig and ear veins have been raised using a rubber band around the ear base. Illustration: Louis Wood.





Figure 1.28 The approach to a post-mortem may differ depending on whether one or many animals have died from a disease. In some cases, sick live animals (Figure 1.29) may need to be euthanized to provide fresh material for laboratory tests. This approach may save other animals and, if the animal cannot be treated, may be considered more humane than leaving it to die (it should be noted, however, that in some cultures this may not be permitted for religious reasons). If an animal is found dead and the carcass is still reasonably fresh a full post-mortem (PM) or necropsy can provide a lot of valuable information. If the carcass has already begun to rot then a quick PM may still be worthwhile but it may not be worth trying to collect a lot of samples. The procedure for performing a PM is outlined in Chapter 8. A simple post-mortem kit is outlined in Table 1.4. Practical limitations as well as expense will often dictate the range of samples taken but usually (unless the cause of death is obvious) the following will be collected: (1) Tissues (healthy and diseased) from the main organ system (lungs, heart, liver, kidney etc.) and tissues demonstrating specific lesions (that is, abscesses, vesicles, ulcers and so on) will be collected and preserved for histological examination (see Chapter 8). (2) Swabs of fluids from lesions and/or tissue samples may be collected for microbiological examination (see Chapter 4). (3) Parasitic organisms (internal and external) (see Chapter 3). (4) Blood may be collected for culture. If anthrax is suspected then you should not open the carcass (see Chapter 4). (5) Specific samples may be collected for specific diagnoses, that is, rumen contents for suspected poisoning (see Chapter 7). (6) Blood/tissue smears for cytology (see Chapter 8). (7) Urine/eye fluid for microbiology (see Chapter 4). Detailed notes should be kept throughout the procedure to describe any abnormalities found during the post-mortem (see post-mortem submission in Appendix A2).



Figure 1.29 Sick animals may need to be euthanized for humane reasons or for disease control. In many cases ante-mortem samples (especially for haematology and serology) from these animals provide the greatest likelihood of making a diagnosis. Perform a thorough clinical examination *before* samples are collected from the animal.



Figure 1.30 Mule with load. In many rural areas, the mule may be the main method of transport. Practical constrains often limit what can be done to treat disease(s) in animals required for the normal day-to-day activities in rural areas. Treatment may be possible for some conditions but practical factors may dictate if and when appropriate treatment is available for administration (for example, antibiotics/pain relief) and it is not always possible to persuade animal owners to follow advice which may cause significant inconvenience (for example, prolonged rest). To get full compliance from the owner of an animal it is essential that the cause(s) of the problem and the nature of the disease(s) present are fully explained and understood. This takes time and requires a good relationship between the extension staff, veterinary officer(s) and animal owner. Illustration: Louis Wood.