SAFETY IN MICROBIOLOGY

edited by

D. A. Shapton and R. G. Board

THE SOCIETY FOR APPLIED BACTERIOLOGY
TECHNICAL SERIES NO. 6

SAFETY IN MICROBIOLOGY

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1972

ACADEMIC PRESS · LONDON · NEW YORK

ACADEMIC PRESS INC. (LONDON) LTD
24/28 OVAL ROAD
LONDON, NW1

U.S. Edition published by
ACADEMIC PRESS INC.
111 FIFTH AVENUE,
NEW YORK, NEW YORK 10003

Library of Congress Catalog Card Number : 77–189937
ISBN : 0–12–638860–1

Printed in Great Britain by
Cox & Wyman Ltd., Fakenham, Norfolk, England

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Preface

THIS volume includes contributions to the Autumn Demonstration Meeting of the Society for Applied Bacteriology, held on 28th October, 1970 at the Department of Biology, Brunel University. It is Number 6 of the Technical Series and it continues the Society's policy of providing workers in a particular field with the opportunity firstly of demonstrating methods and techniques to Members and guests of the Society and secondly of describing these in a book which is intended for use at the bench. The Demonstration had as its central theme, Safety, and the organizers of the meeting adopted a liberal interpretation of this aspect of microbiology so that safe working with chemicals, radioisotopes, *etc.*, is considered along with those techniques which have evolved for routine handling and storing of saprophytic microorganisms and highly virulent pathogens. The liberal interpretation of safety is reflected in this book and it should be of interest not only to microbiologists of long standing but also to the many persons who find employment in microbiology after training in other disciplines where there is perhaps less requirement for the worker to be constantly aware of the risk of cross contamination or infection.

We wish to thank all the demonstrators for the great effort which they took both in the preparation of the exhibits and the chapters in this book.

Our particular thanks go to Professor J. D. Gillett, Mr. F. G. B. Jones, and Mrs. S. Bannerman and other members of the Biology Department of Brunel University for all their help with the laboratory arrangements for the Demonstration.

April, 1972

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Safety in the Microbiological Laboratory: An Introduction

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Since long before the days of Louis Pasteur, workers in the field of pathogenesis have infected themselves in an endeavour to establish those rules that have since crystallized as Koch's Postulates. John Hunter, for example, deliberately and successfully initiated in his own tissues that process, which we now associate with a positive Wassermann reaction, by means, which though relatively painless, were certainly not in the least romantic. It is unfortunate that with increase in knowledge and the availability of *para*-human models laboratory workers still persist in infecting themselves, and more so in that the process is now unintentional; to quote Chatigny and Clinger (1969): "It may be stated without fear of contradiction that every infectious microbial agent which has been studied in the laboratory has, at one time or another, caused infection of operators. In some instances, laboratory infections out-number natural infections and have been the only known human infections." Sulkin (1961) recorded 2348 cases (mainly in the U.S.A.) of presumed laboratory acquired infection with 107 deaths. This may represent little more than the tip of the iceberg, since there is a very natural reluctance to advertise the results of carelessness or ignorance; and many obscure, minor, or subclinical infections must pass undiagnosed, especially when they occur as secondary cases, or in persons not directly concerned in work at the laboratory bench or in the animal house. In yet other instances the etiological connection between a disease and the victim's work may go unrecognized; one wonders how many people died of *Herpes* B virus infections before its connection with a minor mucosal eruption in non-human primates was established. The possibilities are far from being exhausted. New entities, such as Vervet Monkey Disease, may suddenly emerge; old ones, such as Serum Hepatitis, may assume a new significance with changing techniques; and the possible long-term hazards of handling such agents as the oncogenic and slow viruses is now dawning on the scientific conscience. These few examples alone have recently

increased awareness of the need for laboratory safety, about which much has been written, but still relatively little read. This need to be interested should not be a mere function of the anti-pollution movement with its threat of a "Silent Spring", for it is based on the hard fact of morbidity figures which are, in effect, comparable with those of road accidents. Fortunately, there are remedies, albeit often ignored, which can save us from wasteful martyrdom to biological science, risk of justifiable public wrath at potentially dangerous ineptitude, and the "Silent Laboratory".

Pathogens and Non-pathogens

It can be argued that the first step in selecting safe procedures lies in the direction of determining what organisms are pathogenic, and what is the relative infectivity of these for man. Unfortunately, the problem is not as simple as this, as the factors involved are so multitudinous that it could well be simpler to regard **all** microorganisms as presenting some degree of hazard in one way or another. In actual practice, economic considerations all too often dictate the need for, and extent of, precautionary measures, until the occurrence of an expensive failure; though the reverse situation in which work is rendered so intolerably complicated by precautions, often devised from the depths of an armchair, that the worker is tempted to take short cuts, is by no means unknown. A happy medium can be struck only after due assessment of all available considerations, which fall into three main categories, the nature of the organism, the ecosystem of the laboratory (health, hygiene and design) and the mechanics of the experimental techniques employed therein. Let us consider, firstly the organisms. These fall into 7 natural groups.

Established human pathogens

Agents in this category cause conditions ranging from rapidly fatal disease to minor indispositions, or purely localized and self-limiting lesions; they may, or may not, be transmissible to other human contacts, either directly, by vectors, or on fomites. The risk involved in handling them will depend not only on this, but also on viability, virulence, infectivity, portal of entry, size of challenge, the immune status of individuals or populations at risk, and factors specific to laboratory conditions, such as hygiene and experimental procedures; and also on the possession of effective, specific therapeutic measures should the worst occur. The implications of some of these factors will become apparent later, but for the present it must suffice merely to state the obvious—that all established human pathogens should always be handled with circumspection, and preferably with equal caution.

"Avirulent" strains of pathogens

The avirulence of a strain is largely a matter of degree, and is related again to the dose and portal of entry, and to the resistance of individual hosts and host species. A classic example of the latter is that of Strain 19 of *Brucella abortus*, employed as a live vaccine in animals, but which produces florid undulant fever in man. In addition, such events as the accidental substitution of a virulent for an avirulent strain, or enhancement of virulence by mutation or phage transduction must not be ignored. Here again are good reasons for caution.

Pathogens of animals

A very high proportion of pathogens in this category are transmissible to man, some so disastrously that one is inclined to forget that man is not the primary host (e.g. Plague, Tularaemia, Bovine Tuberculosis, and possibly Yellow Fever); whilst of the remainder some are sufficiently horrific (e.g. Rabies, *Herpes* B and Vervet Monkey Disease) to demand extraordinary precautions. Once again there is no excuse for relaxation, and it must not be forgotten that man can, and frequently does, act as an active or mechanical vector of pathogens in animal husbandry, apiary, sericulture, menageries, laboratory animal houses, veterinary practice, and even fisheries.

Plant pathogens

James Thurber stated that his great uncle, Zenas, died in 1866 of the disease that was killing the chestnut trees. Though this claim is unique, not to say dubious, there is no doubt that man is a sufficiently effective mechanical vector of plant pathogens to have stimulated the Ministry of Agriculture to forbid the laboratory handling of a long list of organisms except under licence (Anon, 1965*a*, *b*), issued subject to the provision of satisfactory safeguards. Septicaemia due to *Erwinia* has been reported (Mildvan *etal.*, 1971).

Facultative pathogens

To what extent some strains of *Proteus, Klebsiella, Aerobacter, Escherichia, Paracolobactrum* and *Pseudomonas* are actually primarily pathogenic is still debatable. There can be no doubt, however, that massive and fatal infection can occur in individuals whose normal response has been altered by disease, trauma, irradiation, immuno-suppressive drugs or antibiotics, though in other instances such synergic factors seem to be lacking. Drawing a hard and fast line between safe and unsafe strains in the absence of well

established typing techniques (such as exist fairly comprehensively for Gram positive cocci for example, and, indeed for a few of the above mentioned organisms) is difficult, and one must take into account the source of the organism. Obviously a strain of *Bacillus cereus* or *Serratia marcescens* derived in pure culture from a case of meningitis at *post-mortem* cannot be treated as casually as indistinguishable wild isolates. Such admittedly rare events (and both are recorded) may easily be dismissed as "just one of those things", but could as easily represent an early stage in the evolution of a host/parasite relationship. It is significant, too, that this group of organisms as a whole tend to be poor antigens and resistant to antibiotics. Prophylaxis and therapy, therefore, provide poor defensive prospects.

Into this category, also, must fall organisms which, whilst not pathogenic in themselves, produce toxins under certain abnormal circumstances (e.g. *Clostridium* spp in anaerobic wounds, gut contents, meat pies, etc.), or which promote allergic responses (e.g. the micro-fungi of Farmer's Lung; see p. 151). Conditions of laboratory cultivation generally increase the pathogenic potential of these organisms, and often intentionally as in the production of toxins in toxoid manufacture.

Non-pathogens

Here belong all those organisms which have never started the hare of suspicion running. The list is very long and includes many organisms of major importance in human ecology, such as in sewage disposal, fermentation, nitrogen fixation and antibiotic production. One must be on guard for unexpected complications, but in the main their importance in the laboratory lies in their capacity, if carelessly handled, to create costly contamination problems, particularly as in many cases their relatively simple nutritional requirements and natural resistance to environmental hazards render them difficult to control.

Oncogenic viruses

The association between some viruses and tumour production in experimental and domestic animals is now well established, and, whilst progress in the human field is naturally not so well advanced and almost entirely confined to benign tumours, it is reasonable to expect a similar situation to exist. Malignant lymphomas have, in fact, occurred in laboratory workers involved in research with animal tumour viruses, and, whilst this is still regarded as coincidental, this and similar episodes have been sufficiently disquieting to have prompted the U.S. Department of Health to issue a code of practice on the safe handling of tumour viruses and cells (Anon,

1970). In the same document references are given to aerosol transmission of oncogenic viruses, excretion in urine and faeces of laboratory animals, and the detection of antibodies in the serum of laboratory staff. It is clear, therefore, that the laboratory ecology of at least some oncogenic viruses differs in no way from that of other types of infective agent.

A property of some viruses which may be relevant to safety is that of bimodal expression. Several viruses, including adenovirus strains of human origin, have been found to produce tumours in animals under certain laboratory conditions (e.g. in very high dosage in suckling mice). Spontaneous cases of this phenomenon are known in short-lived animals, but there is no clear evidence to suggest extension of the principle to humans. Nevertheless, it is probably fortunate that the organisms are already identified with established or "avirulent" pathogens. Until proved otherwise, therefore, it must be assumed that oncogenic animal viruses may constitute an oncogenic hazard to man, though the picture is complicated by the discovery that a virus infection can be expressed in different hosts by entirely different pathology.

The Mechanism of Infection

A more detailed attempt to categorize the contents of Bergey's Manual would only result in a process referred to by the Oxford English Dictionary as "floccinaucinihilipilification", and would be less meaningful. Nosological breakdown tables of human laboratory infections have appeared frequently in the literature which, judging from the absolute numbers of casualities, suggest that certain agents, notably *Francisella tularensis, Brucella* spp and *Coxiella burnetti,* are more infective than others. Whilst these assessments are probably close to the truth, they do not take into account variables such as the number of persons at risk, the relative hazards of the different handling techniques employed, and local hygiene or medical factors, and it may well be that more rarely handled agents are equally infective. The argument has, therefore, turned full circle to the original contention that most, if not all, microorganisms commonly handled in laboratories should be treated with respect for reasons ranging from their lethal potential to their nuisance value. Nevertheless, some ground has been cleared, and it can at least be said that the maximum hazard lies in handling potential killers and material of unknown potential sent to diagnostic laboratories (Sulkin *et al.*, 1963) for investigation.

The next logical step in assessing risk and how to reduce it is to consider transmission. This is divided into two stages: (a) dissemination, (b) invasion, or cause and effect; and as it is the latter that provides evidence that the former has occurred, it is to this that attention must first be

directed, particularly in respect to human laboratory populations. Under natural conditions pathogens enter the human body through discontinuities in the skin, including all kinds of wound, insect bites, burns, blisters and dermatological conditions, by direct invasion of the mucosal surfaces of the gastrointestinal tract (i.e. by ingestion), mouth, nasopharynx, eyes and lower urogenital tract. Only in exceptional circumstances does the lower respiratory tract constitute a primary portal of entry, most pulmonary infections being secondary to systemic infection or downward extension from the throat. Of the exceptions the three classic examples are pulmonary tuberculosis, pneumonic plague and inhalation anthrax, in which the predisposing common factor is overcrowding in insanitary, ill-ventilated spaces, where the risk of inhaling infective material is greatly increased (see p. 167). This factor is also common to many laboratories, where all three examples have in fact taken their toll.

It has been found repeatedly that only a minority of recorded human laboratory infections are preceded by an overt accident, of which few have involved aerosol dissemination; the remainder of such cases followed accidental hypodermic inoculation or ingestion during mouth pipetting, and similar episodes of which a worker is conscious. Nevertheless, it has been concluded by Sulkin (1961) and other specialists in this field that in cases unassociated with an accident (approximately 80%) the prelude has been unsuspected aerosol generation during ordinary laboratory procedures. This does not imply pulmonary or even pharyngeal pathology, as in the case of plague, etc., since the tissue preference of many pathogens does not necessarily involve this, but animal and both accidental and intentional human challenge have adequately demonstrated that the great majority of pathogens can gain access by the pulmonary, or at least respiratory, portal of entry regardless of their natural mode of transmission. The arboviruses (Hanson *et al.*, 1967) provide an outstanding example of this.

Almost all common laboratory bench techniques and accidents produce aerosols in varying degrees, and the literature on the subject is, in fact, voluminous. References to it are given by Kenny and Sabel (1968) who confirmed previous findings and determined the concentrations and particle size ranges of aerosols generated during common procedures and simulated accidents. Infective laboratory aerosols can be divided more or less neatly into three categories.

Droplet nuclei

When disruptive stresses are applied at the surface of a liquid, droplets are detached. In laboratory air these evaporate extremely quickly (Green and

Lane, 1964), leaving nuclei of suspended and dissolved solids. The smaller the solid content of the original liquid, then the smaller will be the resultant nuclei for any given droplet size. The smaller the nuclei, the longer will they remain airborn (Green and Lane, 1964) and the greater their chances of dissemination and of being inhaled. Furthermore, the smaller the particle, the deeper into the respiratory tract can it penetrate (see p. 167). Much has been published in the last quarter of a century on this latter aspect which demonstrates that at least some agents can infect in smaller dosage and with more drastic results and shorter incubation periods, when the particle diameter is less than 5 μ and pulmonary penetration achieved, than when larger and retained in the nose (see p. 167). This suggests that pipetting serial dilutions of bacterial suspensions and subsequent plating out, when accompanied by careless splashing and frothing, is particularly hazardous. Casualty figures tend to confirm this, though the manipulation of relatively viscous slurries (e.g. pus, blood, sputum, infected tissue cultures, etc.) is by no means free from risk (Kenny and Sabel, 1968).

Dried materials

The disintegration of lyophilized cultures, crusts on stoppers and screw-caps, dry colonies and exudates, and the dehiscence of fungal sporangia are all potent sources of small, light particles with an unusually concentrated content of infective units. It can also be shown that the act of cutting deep-frozen tissue sections liberates minute fragments of material that are widely disseminated and may also present a bio-hazard.

Vector dusts

These consist of fur, down, skin scales, cage litter, "fluff" (e.g. cotton wool plugs and hospital blankets) and heterogeneous particulate detritus usually dignified as house dust. The particles are relatively large, but buoyant. Their presence is readily observed, and accumulations are surely a sign of bad housekeeping. They may be contaminated either at source or by subsequent fall-out of smaller particles. Whilst they may well constitute a lesser respiratory hazard than the foregoing groups in some respects, because of the ease with which they are filtered out in the nasal passages, there is little doubt that they can contribute to the transmission of disease as exemplified by cage and hospital cross-infection and in the spread of certain epizootic diseases.

The infective potential of these three classes of particle depends not only on the factors already mentioned, but also upon the viability of the contained organisms. Apart from physical influences such as ambient