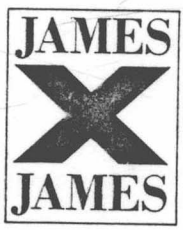
Immunoassays for Food-poisoning Bacteria and Bacterial Toxins

G. M. Wyatt

with contributions from
H. A. Lee and M. R. A. Morgan
AFRC Institute of Food Research, Norwich, UK



CHAPMAN & HALL

London · Glasgow · New York · Tokyo · Melbourne · Madras

Co-published by James & James (Science Publishers) Ltd, 5 Castle Road, London NW1 8PR and Chapman & Hall, 2–6 Boundary Row, London SE1 8HN

Chapman & Hall, 2–6 Boundary Row, London SE1 8HN, UK

Blackie Academic & Professional, Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ, UK

Van Nostrand Reinhold Inc., 115 5th Avenue, New York NY10003, USA

Chapman & Hall Japan, Thomson Publishing Japan, Hirakawacho Nemoto Building, 6F, 1-7-11 Hirakawa-cho, Chiyoda-ku, Tokyo 102, Japan

Chapman & Hall Australia, Thomas Nelson Australia, 102 Dodds Street, South Melbourne, Victoria 3205, Australia

Chapman & Hall India, R. Seshadri, 32 Second Main Road, CIT East, Madras 600 035, India

First edition 1992

Typeset in 12/13pt Garamond 3 by Columns of Reading Ltd
Printed in Great Britain

ISBN 0 412 408104 0 442 316542 (USA)

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

Library of Congress Cataloging-in-Publication data available

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Series Introduction

Consumer safety has become a central issue of the food supply system in most countries. It encompasses a large number of interacting scientific and technological matters, such as agricultural practice, microbiology, chemistry, food technology, processing, handling and packaging. The techniques used in understanding and controlling contaminants and toxicity range from the most sophisticated scientific laboratory methods, through industrial engineering science to simple logical rules implemented in the kitchen.

The problems of food safety, however, spread far beyond those directly occupied in food production. Public interest and concern has become acute in recent years, alerting a wide spectrum of specialists in research, education and public affairs.

This series aims to present timely volumes covering all aspects of the subject. They will be up-to-date, specialist reviews written by acknowledged experts in their fields of research to express each author's own viewpoint. The readership is intended to be wide and international, and the style to be comprehensible to non-specialists, albeit professionals.

The series will be of interest to food scientists and technologists working in industry, universities, polytechnics and government institutes; legislators and regulators concerned with the food supply; and specialists in agriculture, engineering, health care and consumer affairs.

One of the most difficult situations to control is the contamination of food by small numbers of pathogenic micro-organisms before they multiply to give the large populations causing food poisoning when eaten.

A rapid detection method would be of immense value to food producers and retailers, to public analysts and legislators as well as benefiting the public at large at a time when the reported incidence of food poisoning appears to be rising year by year. This book, by Drs Wyatt, Lee and Morgan, describes the current state of research into the powerful and elegant techniques of immunoassay as a means of dealing with this microbiological problem.

J. Edelman

Acknowledgement

The authors wish to acknowledge the Photography Section at the Institute of Food Research, Norwich for excellent preparation of the figures, and Mrs Janet Arnett for careful typing of the manuscript.

Preface

This book is intended to be a compilation or distillation of current knowledge of the subject, and most of the material in it is within the experience of the authors. Where necessary, specific references to the literature have been made; otherwise a bibliography is given at the end of the appropriate chapter.

In order not to interrupt the flow of the text, detailed methodology has been consigned to a series of appendices.

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—1—

Introduction

At the end of the 1980s, public awareness of the possible presence of food-poisoning bacteria in the food supply of the developed countries greatly increased. Examples receiving much publicity in the United Kingdom included outbreaks of salmonellosis originating from unprocessed eggs and the emergence in the public eye of *Listeria monocytogenes* as a potential pathogen (Figure 1.1).

In contrast to many other aspects of life in the Western World, where considerable improvements had been made in the quality of life, the contemporary diet came to be seen as a potential cause of morbidity and mortality. Although, in the home and workplace there had been a great deal of newly introduced technology, the methods of food analysis for detecting these pathogens were, in the 1980s, still firmly rooted in the 1880s, relying on ideas developed by the great pioneers of bacteriology such as Koch, Pasteur and Lister.

Clearly, in the public perception these methods were failing to detect pathogens in food or, more likely, were never actually being applied by the food producer, largely, it would seem, due to the cumbersome, labour-intensive and time-consuming techniques involved. However, in 1989, in response to public concern, the United Kingdom launched an investigation into the microbiological safety of food (HMSO, 1990, 1991); this was followed by passage of a Food Safety Bill through Parliament and this, together with other regulations such as the Poultry Orders, imposed additional responsibility on food

Figure 1.1
Food poisoning hits the headlines

producers to ensure that food destined for human consumption is not 'injurious to health'. The incentive for the considerable increase in testing of foods required by this legislation is blunted by the problems of traditional technology; if foods are to be certified as pathogen free (an impossible ideal?), then several days of expensive cold storage would be required while testing proceeded. Clearly, methods that are both faster and more convenient are required if producers are to fulfil their obligations. What happens to the food in the hands of the consumer is, of course, beyond the control of the producer and is largely a matter of public education.

Many approaches to the development of rapid methods have been taken, but for specific detection of pathogens the leading technology in the field is based on the use of antibodies in immunoassays. Relatively

rapid methods for the total count of bacteria in a food sample have been available for several years, such as the direct epifluorescent filter technique (DEFT) and methods based on changes in electrical conductance or impedance in a culture medium during growth of bacteria. However, these methods are unable to discriminate easily between closely related genera of bacteria and are thus unsuitable for specific pathogen detection.

Immunoassays, in addition to their specificity and sensitivity, have another great advantage in that many formats are available. It should be stated here that, in the present context, the term 'immunoassay' might be replaced by 'immunotechnique' because some of the concepts to be covered in this book are not assays in the strict sense. The most widely used format at present in the non-clinical area is the enzyme-linked immunosorbent assay (ELISA). This can be based on a 96-well microtitration plate, the flexibility of which allows for analyses that are completely manual, completely automated or any stage in between. Enzyme immunoassays were developed from radio-immunoassays originally described by Yalow and Berson (1959) in which isotopes were used for end-point detection. Radio-isotopes present problems of safe disposal and, additionally, are not seen as acceptable in a food laboratory. An alternative, non-isotopic, end-point detection system was required and, in 1971, the ELISA, in which one component is immobilized and enzymically mediated colour reactions act as the end-point, was introduced (Engvall and Perlmann, 1971; van Weeman and Schuurs, 1971). A further important development came with the introduction of monoclonal antibodies (Kohler and Milstein, 1975) which have much greater potential specificity and which could theoretically be produced in almost unlimited quantities *in vitro*. ELISAs still took time to become accepted in the food analysis laboratory, where traditional methodology seems to have greater inertia than in the clinical laboratory. However, commercially available ELISAs for food-poisoning bacteria or their toxins do now seem to have been accepted and are becoming more widely used: some have received official approval as analytical methods. Other test systems based on the use of antibodies are emerging.

It is hoped that this book will provide the reader with at least two things: first, sufficient background to understand the theory behind any commercial assays used in their laboratory and to enable these to be

adapted if necessary and validated for a particular use; secondly, to contribute, perhaps, to expansion of the field by developing assays for analytes not currently available. The authors have gained much experience in this area and are eager to pass it on!