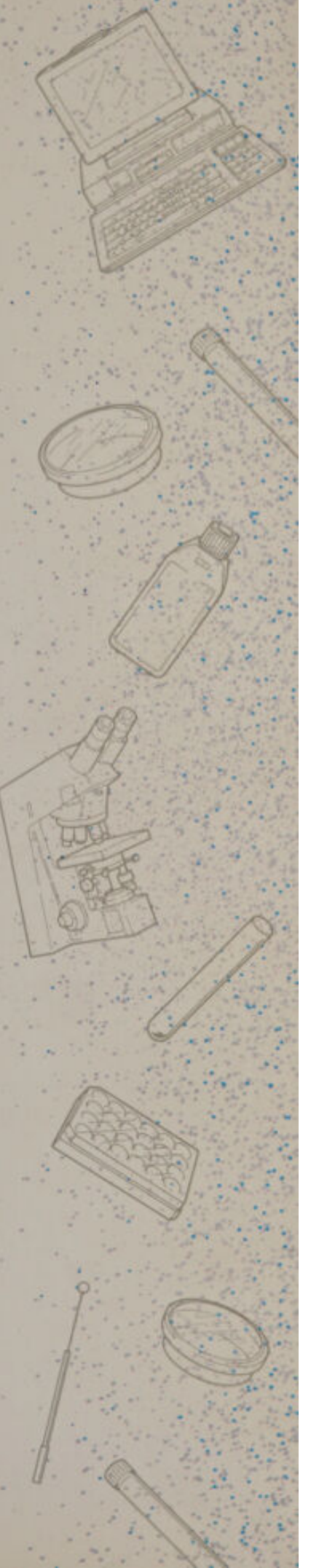
**Public Health Laboratory Service / prepared by the Central Office of Information for the Public Health Laboratory Service.**



PHLS

PUBLIC HEALTH
LABORATORY SERVICE

# CENTRAL PUBLIC HEALTH LABORATORY

CPHL

PUBLIC HEALTH LABORATORY SERVICE

# FUNCTIONS

The Central Public Health Laboratory is the principal reference centre for microbiology in the UK. Part of the Public Health Laboratory Service, it provides specialist advice and assistance to NHS hospital laboratories, as well as to the 52 area and regional PHLS laboratories. In addition, community and hospital physicians, environmental health officers, local and central government, industry, and others regularly consult the CPHL.

Expert divisions within the CPHL specialise in particular groups of micro-organisms or areas of microbiology. By using sophisticated identification systems, including molecular techniqes, the CPHL can offer a range of subtyping services for a wide variety of organisms. Many other expert investigations are also available.

Through its reference role the CPHL provides an unparalleled body of microbiological data that is constantly adding to our knowledge of the micro-organisms that cause infectious disease – their origin, how they spread and how they can be detected. This knowledge is applied by the CPHL in its research into better methods of controlling and preventing human infectious disease and the development of faster, more convenient and accurate diagnostic tests.

# STRUCTURE

**DIRECTOR: DR. MORAG TIMBURY**

**Deputy Director: Dr. Richard Gilbert**

**DIVISION OF ENTERIC PATHOGENS**
Director: Dr. Bernard Rowe

**FOOD HYGIENE LABORATORY**
Director: Dr. Richard Gilbert

**DIVISION OF HOSPITAL INFECTION**
Director: Dr. Barry Cookson

**DIVISION OF MICROBIOLOGICAL REAGENTS**
Director: Dr. Tony Taylor

**MYCOLOGICAL REFERENCE LABORATORY**
Director: Professor Donald Mackenzie

**NATIONAL COLLECTION OF TYPE CULTURES**
Director: Dr. Rowland Hill

**QUALITY ASSURANCE LABORATORY**
Director: Dr. Jerry Snell

**VIRUS REFERENCE LABORATORY**
Director: Dr. Philip Mortimer

**ADMINISTRATION**
**MEDIA PRODUCTION**
**BIOLOGICAL SERVICES**
**LIBRARY**
**MEDICAL ILLUSTRATION**

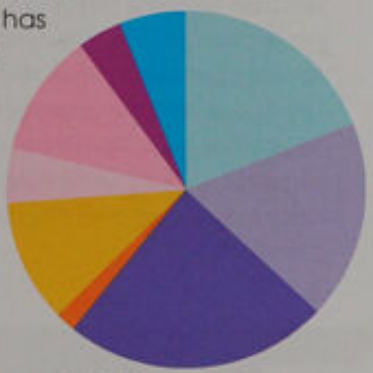
The work of the CPHL enjoys international recognition with several World Health Organisation collaborating centres being based in its laboratories. It also has a number of joint projects underway with colleagues in Europe, America, Africa, Asia and Australasia.

# RESOURCES

Over 400 people work at the CPHL:

- MEDICAL 17
- CLINICAL SCIENTISTS 119
- MEDICAL LABORATORY SCIENTIFIC OFFICERS 106
- SERVICES AND SUPPORT STAFF 100
- ADMINISTRATION 73

It has annual budget of around £10 million; this is divided between the various activities as shown below. A proportion of its income is generated by the manufacture of a range of diagnostic reagents and kits. The UK National External Quality Assessment Scheme for Microbiology (NEQAS) has been administered for many years now by the CPHL – this scheme is open to both UK and overseas laboratories providing clinical diagnostic services.



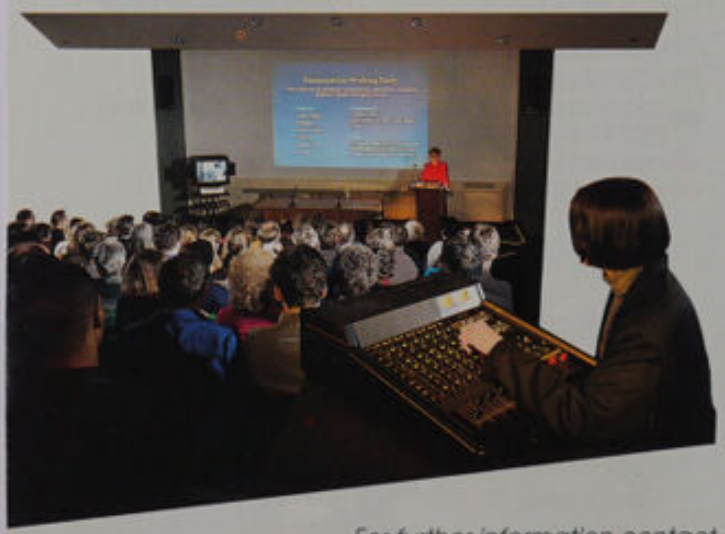
- VIRAL DISEASES
- HOSPITAL INFECTIONS
- FOODBORNE AND ENTERIC INFECTION
- LEGIONELLA REFERENCE
- REAGENT PRODUCTION
- NEQAS
- NATIONAL COLLECTION OF TYPE CULTURES
- FUNGAL DISEASES
- AIDS RESEARCH

The CPHL also enjoys one of the best microbiological reference libraries in the country with over 7,500 volumes and around 450 journals regularly received.

# FACILITIES

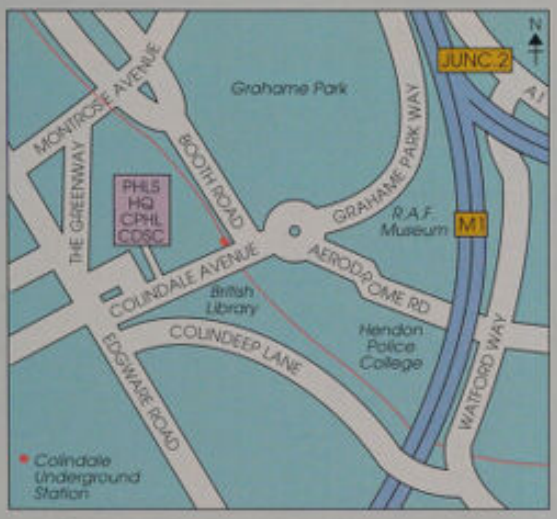
The CPHL is based in large, modern laboratories located in the northern outskirts of Central London. It is readily accessible by road, and both bus and tube services are only a few minutes walk away. It has a comfortable refectory, administered by a firm of professional caterers. As well as a daily subsidised canteen menu, full conference catering is available.

A small complex of conference facilities is available on site. These consist of the Wilson Lecture Theatre which seats 174 and has full projection and audio-visual equipment. Adjacent to this, and just off the main reception and cloaks area, are two seminar rooms which can be fitted to various specifications. This small complex is in regular use for both internal PHLS conferences and workshops and also for external scientific meetings.



*For further information contact*
*Mr. Melvyn Danvers. Telephone: 081 200 4400 ext 4940.*

# LOCATION




Central Public Health Laboratory
61 Colindale Avenue
London NW9 5HT
Telephone: 081-200 4400
Fax: 081-200 7874

The Public Health Laboratory Service is a network organisation consisting of 52 laboratories strategically located throughout England and Wales which are linked to specialist microbiological reference units and epidemiological experts. The constant flow of microbiological information through this national network provides a unique perspective for the detection of outbreaks of infectious disease and the identification of emerging patterns or trends in human infection in the country.

# THE PHLS WATER MICROBIOLOGY EXTERNAL QUALITY ASSESSMENT SCHEME


The Public Health Laboratory Service (PHLS) is a network of 52 microbiology laboratories strategically distributed throughout England and Wales, which are linked to central specialist microbiological reference and epidemiological facilities. Its remit is the diagnosis, prevention and control of human infections and communicable diseases. Every day this network examines over 25,000 human clinical specimens and environmental samples. This level of throughput places the PHLS in the forefront of laboratory methodology and has led to its involvement in the setting up and operation of a number of external quality assessment schemes. The PHLS Water Microbiology External Quality Assessment Scheme first began as an experimental internal PHLS scheme in 1984. Its users now number 170 laboratories from not only the PHLS but also from Water Supply Companies, the Ministry of Agriculture, Fisheries and Food, Public Analysts, Environmental Health Departments, research organisations, the private sector and Europe.

## Measurement of Laboratory Proficiency

The standards set in the European Directives and the Water Supply (Water Quality) Regulations 1989 define the wholesomeness of water and set high standards: indicator organisms must be absent in 100ml volumes of water. These stringent standards require stringent sampling and laboratory procedures. A full quality assurance programme thus becomes an absolute requirement. This should not be confined merely to quality control of the technical examination to which a sample is subjected in the laboratory but should also encompass sample collection and transportation, and interpretation of results. The final element in any such quality assurance programme should be the participation in an external quality assessment (QA) scheme. Membership of such a scheme gives the laboratory an independent measure of performance against which to assess its own internal quality control measures. It also provides valuable management information for the more specific targetting of training needs and laboratory resources.

## The PHLS Water Microbiology External QA Scheme

PHLS expertise in water microbiology, as well as the diagnosis and epidemiology of human water-borne infections, is well-recognised. In recent years, PHLS advice has been sought by several government committees examining the safety of both recreational waters and water supplies. In 1991 a special PHLS Water and Environmental Microbiology Laboratory was set up, and PHLS medical and scientific staff participated in a series of seminars organised by the Department of Health on 'Public Health Aspects of Drinking Water Supplies'. This breadth of expertise, together with its many years experience

of operating a QA scheme for laboratories providing clinical diagnostic services - the UK National External Quality Assessment Scheme for Microbiology (NEQAS), placed the PHLS in a unique position to develop similar proficiency measurement for water microbiology. Since its inception in 1984 at the Newcastle upon Tyne Public Health Laboratory, the PHLS QA scheme for water indicator organisms has continued to grow and develop. Its research basis is considerable and on-going; a QA scheme for cryptosporidium in water will be available shortly and work on a pilot scheme for water virus QA is underway.

## Participation in the Scheme

Membership is open to any bona fide laboratory involved in water microbiology - in either the private or public sector. Participating laboratories will be required to register for a minimum of one full year. There will be an annual charge of £500 for each laboratory registered. Six distributions of simulated waters are made each year, participants receive three samples in bijou bottles through the post and are asked to examine them on a set date. The samples are diluted to 400 ml volumes and should be processed as routine samples. Five distributions cover low levels of coliforms, *Escherichia coli*, and faecal streptococci and one distribution simulates the higher levels of contamination seen in raw surface waters. There will be two to three distributions for the cryptosporidium scheme; an additional charge will be made to those laboratories taking them. The returned results are collated, analysed and a comprehensive report produced. The PHLS recognise the problems of the variable distribution of bacteria in water and has therefore developed a statistical marking scheme which takes this into account and provides short-term and long-term *cumulative* performance assessments.

## Please Send Me Further Information on the PHLS Water Microbiology Quality Assessment Schemes

- ☐ I am already a participant in the PHLS indicator organisms scheme
- ☐ I am interested in the indicator organisms scheme
- ☐ I am interested in the cryptosporidium scheme
- ☐ I am already a participant in the WRC Aquacheck scheme
- ☐ I am interested in the WRC Aquacheck scheme

Name _____

Organisation _____

Position _____

Address _____

_____

_____

_____

Please enclose an example of your organisation's letter-headed paper

## Confidentiality

Analysis of results is communicated by the PHLS to each participating laboratory in strict confidence and is not revealed to third parties by the PHLS. Participants are free, of course, to divulge their own QA results to whomsoever they wish. Confidential consultant and technical advice is also available from the PHLS for those participating laboratories who wish to discuss their results.

## Further Information

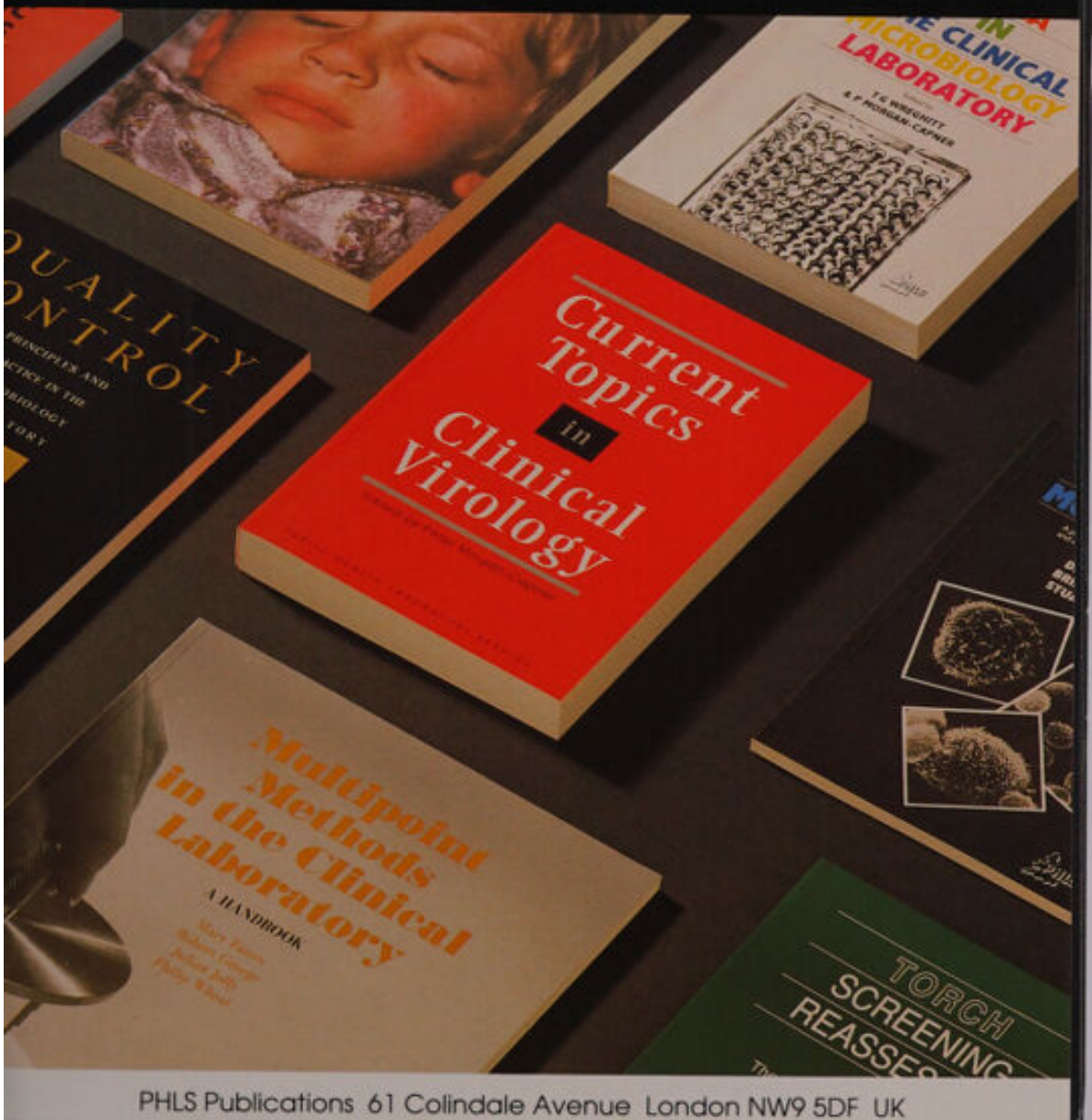
If you or your organisation are interested in receiving further details of the indicator organisms scheme or the cryptosporidium scheme, please complete and return the tear-off slip to the address below. To provide a more comprehensive service for those in the water industry, we hope to establish closer collaborative ties with the Water Research Centre and their water chemistry QA scheme. Therefore, we would both find it helpful to learn whether you currently participate, or are interested in participating, in the WRC Aquacheck scheme.

## To:

Public Health Laboratory
Institute of Pathology
General Hospital
Westgate Road
Newcastle upon Tyne NE4 6BE
United Kingdom

PUBLIC HEALTH LABORATORY SERVICE

# Publications Catalogue




PHLS Publications 61 Colindale Avenue London NW9 5DF UK

# HOW TO ORDER

Please enclose payment with your order and send it to

The Treasurer
Public Health Laboratory Service
61 Colindale Avenue
London NW9 5DF
UK

## Methods of Payment

Cheques or money orders should be in sterling and made payable to the Public Health Laboratory Service. Orders can only be accepted on a cash-with-order basis. Receipts are not normally issued unless requested.

## Postage and Packing

Prices quoted include postage and packing in the UK. Overseas purchasers should add £2 sterling per order. Orders are despatched as soon as possible after receipt of payment.

## Enquiries

Enquiries, trade orders and requests for additional catalogues or a full backlist should be directed to PHLS Publications, 61 Colindale Avenue, London NW9 5DF, UK (tel 081-200-1295, fax 081-200-8130/8131).

# ORDER FORM 1992

## BOOKS

Payment must accompany your order. Please make cheque or money order (sterling only) payable to Public Health Laboratory Service. Prices include postage and packing to UK addresses. Outside the UK please add £2 per order.

| Title | Quantity | Price | Total |
|---|---|---|---|
| Anaerobic Infections | | £20.00 | |
| Current Topics in Clinical Virology | | £25.00 | |
| ELISA in the Clinical Microbiology Laboratory | | £20.00 | |
| Hygiene for Hydrotherapy Pools | | £5.00 | |
| Making Monoclonals | | £10.00 | |
| Multipoint Methods in the Clinical Laboratory | | £6.95 | |
| PHLS Directory 1991-92 | | £4.50 | |
| Public Health Virology | | £15.00 | |
| Quality Control | | £12.50 | |
| Sexually Transmitted Diseases | | £10.00 | |
| TORCH Screening Reassessed | | £6.50 | |
| | Postage outside UK | | £2.00 |
| | **Total enclosed £** | | |

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Address _____

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Postcode _____

Signature _____ Date _____

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# ORDER FORM 1992

## SERIAL PUBLICATIONS

New subscriptions and renewals

Payment must accompany your order. Please make cheque or money order (sterling only) payable to Public Health Laboratory Service.

**I would like a one-year subscription to:**

Microbiology Digest

I enclose ☐ £16 (UK) ☐ £19 (Europe) ☐ £24 (Elsewhere)

Library Bulletin

I enclose ☐ £45 (UK) ☐ £78 (Europe) ☐ £110 (Elsewhere)

HIV Bulletin

I enclose ☐ £34 (UK) ☐ £41 (Europe) ☐ £56 (Elsewhere)

**I would like a combined one-year subscription to:**

Microbiology Digest and Library Bulletin

I enclose ☐ £55 (UK) ☐ £88 (Europe) ☐ £120 (Elsewhere)

Microbiology Digest and HIV Bulletin

I enclose ☐ £44 (UK) ☐ £51 (Europe) ☐ £66 (Elsewhere)

Microbiology Digest, Library Bulletin and HIV Bulletin

I enclose ☐ £80 (UK) ☐ £115 (Europe) ☐ £148 (Elsewhere)

Name _____

Address _____

_____

_____

Postcode _____

Signature _____ Date _____

TO: THE TREASURER, PUBLIC HEALTH LABORATORY SERVICE, 61 COLINDALE AVENUE, LONDON NW9 5 DF, UK

# COMMUNICABLE DISEASE REPORT

PHLS Communicable Disease Surveillance Centre
61 Colindale Avenue, London NW9 5EQ
Tel: 081 200 6868, Fax: 081 200 7868, Telex: 8953942


Since the Public Health Laboratory Service first published the Communicable Disease Report (CDR) in 1963 it has provided those involved in the prevention and control of human infection with a weekly analysis of microbiological and infectious disease data together with reports of current interest. In January 1991 the CDR became a subscription publication available to all. The CDR is published in two forms: a weekly bulletin and a four-weekly review (the CDR review).

The **weekly bulletin** contains summarised data, derived from laboratory reports in England and Wales, notifications of infectious disease from the Office of Population, Censuses and Surveys, and other surveillance systems such as general practitioner sentinel programmes. News items and current problems form the basis of the front page of each issue. Each calendar month, data on AIDS and HIV infection in the United Kingdom are included.

The **CDR review** contains original and review articles, outbreak reports and other information concerning communicable diseases. Topics of European and international importance are also featured.

The CDR is an essential tool for all those concerned with communicable disease control, both in the United Kingdom and internationally. Regular readership includes microbiologists, consultants in communicable disease control, environmental health officers, public health physicians, clinicians involved in infectious diseases, those concerned with food, water and veterinary aspects of infection, relevant academic departments and representatives of health departments and agencies in the United Kingdom and overseas.

## ORDER FORM

Name _____ Title _____

Position _____

Department/Institution _____

Address _____

_____ Postcode _____

My primary specialty (or field of interest) is _____

Signature _____ Date _____

Please complete reverse side

**TO: EDITOR CDR, PHLS COMMUNICABLE DISEASE SURVEILLANCE CENTRE, 61 COLINDALE AVENUE, LONDON NW9 5EQ**

### Policy of charging

Those people who report data regularly to the PHLS Communicable Disease Surveillance Centre will receive the CDR without charge. The annual subscription will be waived for recipients in the following categories:

(a) Public Health Laboratories, one copy for each of the consultants and scientists of top grade level or equivalent; plus two additional copies.
(b) Other reporting microbiology laboratories, two copies.
(c) Consultants in Communicable Disease Control, one copy each.
(d) Directors of Public Health, one copy each.
(e) Environmental Health Departments, two copies.
(f) Armed forces, two copies per laboratory.
(g) Paediatricians participating in the British Paediatric Surveillance Unit, and genito-urinary medicine physicians collaborating with CDSC in surveillance projects, one copy each.
(h) General physicians, infectious disease physicians, chest physicians, dental practitioners and other clinicians contributing data to CDSC, one copy each.
(i) Veterinary Investigation Centres, two copies each.
(j) Appropriate university and polytechnic departments, one copy each.
(k) Department of Health, England, the Welsh Office, Ministry of Agriculture, Fisheries and Food, DHSS N. Ireland, Scottish Home and Health Department, Communicable Diseases (Scotland) Unit, MAFF Central Veterinary Laboratory, the Home Office, Office of Population Censuses and Surveys, Royal College of General Practitioners Research Unit and British Paediatric Surveillance Unit.

Distribution outside the UK and the Republic of Ireland: the CDR will be sent without charge only to national surveillance centres and ministries/departments of health.

Enquiries concerning this policy should be directed in writing to the Editor.

### Subscription rate

The subscription rate (£65 sterling for 1992) is the same for individuals, libraries and institutions, whether in the United Kingdom or overseas. Airmail will be used for overseas copies.

### Payment

For annual subscription(s) please enclose cheque or money order (in £ sterling) drawn on a UK bank, payable to "Public Health Laboratory Service". Amount should be £65 multiplied by the number of subscriptions requested.

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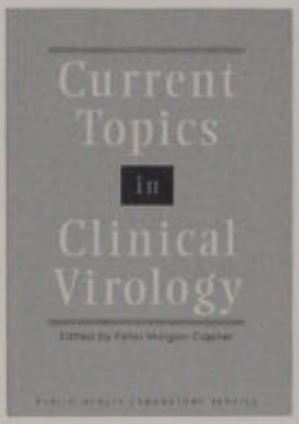
## Current Topics in Clinical Virology

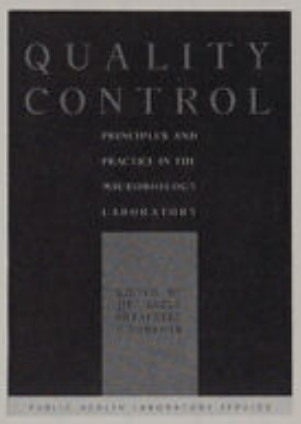
*Edited by* ***Peter Morgan-Capner***
*Consultant Virologist, Royal Preston Hospital,*
*Honorary Consultant Medical Microbiologist,*
*Preston Public Health Laboratory*




1991 ISBN 0 901144 30 4 Paperback
148x210mm 304pp £25

The 14 reviews in this book reflect the broad scope and complexity of modern diagnostic virology. Chapters on epidemiologically and clinically important infective agents such as *Chlamydia trachomatis, Coxiella burnetii* and *Mycoplasma pneumoniae*, often overlooked in such volumes, are included here since they are most frequently diagnosed in the virology laboratory. Recent developments in the growing field of water virology are also discussed, while other chapters provide a current overview of topics which will be of interest to anyone working in clinical virology.

- Varicella-zoster virus infection during pregnancy
- Human viruses and water
- Mumps - worthy of elimination?
- Norwalk-like viruses and winter vomiting disease
- HTLV-1
- The diagnosis of Epstein-Barr virus-associated disease
- Rabies - recent developments in research and human prophylaxis
- Treatment of cytomegalovirus infections
- Hantavirus
- Oculogenital Chlamydia trachomatis infections and their diagnosis
- Chronic Q fever
- Mycoplasma pneumoniae
- Microbiology in the National Blood Transfusion Service
- Quality assessment in virology




## Quality Control
Principles and Practice
in the Microbiology Laboratory

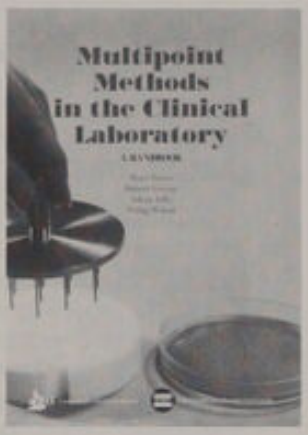
*Edited by **JJS Snell**, Director,*
*Quality Assurance Laboratory,*
*Central Public Health Laboratory*
***ID Farrell**, Director,*
*Birmingham Public Health Laboratory*
*East Birmingham Hospital*
***C Roberts**, Deputy Director,*
*Public Health Laboratory Service*

1991 ISBN 0 901144 31 2 Paperback 148x210mm 158pp £12.50

Quality control and assessment are essential aspects of laboratory medicine. This manual sets out the principles of quality control and provides clear, detailed information on the practical ways in which good quality control can be achieved and maintained in the microbiology laboratory.

- External quality assessment
- Culture media
- Bacteriological characterisation tests
- Antibiotic susceptibility testing
- Antibiotic assays
- Preservation of control strains
- Immunoassays
- Virus isolation
- Electron microscopy
- Mycology
- Parasitology
- Water microbiology
- Food microbiology - a PHLS perspective
- Food microbiology - an industrial perspective

## Multipoint Methods in the Clinical Laboratory
A Handbook




***Mary Faiers**, Senior Medical Microbiologist, Luton Public Health Laboratory*
***Robert George**, Consultant Medical Microbiologist, Division of Hospital Infection, Central Public Health Laboratory*
***Julian Jolly**, Senior Chief Medical Laboratory Scientific Officer, West Suffolk Hospital, Bury St Edmunds*
***Philip Wheat**, Senior Chief Medical Laboratory Scientific Officer, Royal Hallamshire Hospital, Sheffield*

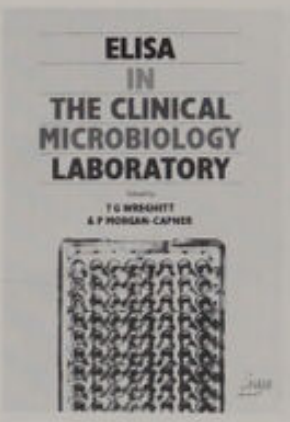
1991 ISBN 0 901144 28 2 Paperback 210x298mm 95pp £6.95

This book is designed as a guide to the principles and practice of multipoint inoculation in the diagnostic laboratory. The authors review the development of multipoint instrumentation and assess its practical use and efficacy in various applications. They also provide comprehensive tables, including breakpoint recommendations for antimicrobial susceptibility testing and the identification of Enterobacteriaceae and staphylococci.

- Historical review
- Antimicrobial susceptibility testing
- Identification of Enterobacteriaceae
- Identification of Gram-positive organisms
- Multipoint techniques for urine specimens
- Additional multipoint applications
- Application of computers, replicating and reading devices to multipoint technology

Published jointly by the Public Health Laboratory Service and the British Society for Microbial Technology

*'... a full account of the techniques for multipoint inoculation. ... for anyone contemplating leaving discs and commercial ID kits behind, this book needs to be read first.'*
Journal of Hospital Infection




## ELISA in the Clinical Microbiology Laboratory

*Edited by* ***TG Wreghitt,***
*Acting Deputy Director,*
*Cambridge Public Health Laboratory*
***P Morgan-Capner****, Consultant Virologist,*
*Royal Preston Hospital;*
*Honorary Consultant Medical Microbiologist,*
*Preston Public Health Laboratory*

1990 ISBN 0 901144 24 X Paperback
148x210mm 305pp £20

Enzyme-linked immunosorbent assays were first described in the early 1970s. Since then ELISAs have become widely used in qualitative and quantitative diagnostic tests for bacteria and viruses. They can be simpler, more easily automated, more sensitive and more specific than traditional alternatives, but not always: success requires a close knowledge of when an ELISA is appropriate and how exactly to apply it. This book gives clear, direct accounts - drawn from practice, with discussion of actual results - of ELISAs applied to the diagnosis of a wide range of infections. The book's 25 distinguished authors - drawn from outside and inside the PHLS - evaluate realistically the merits of ELISA and other methods for the clinical microbiology laboratory.

*'... a wealth of technical detail, together with protocols for ELISA procedures, detailed instructions for the production of antisera and comments that clearly derive from personal bench experience.'*

Abstracts on Hygiene and Communicable Diseases

*'It is remarkable that in each subject the accurate description of the technical protocols is associated with the discussion of the specific diagnostic problems and the interpretation of the results.'*

Society for General Microbiology Quarterly

## TORCH Screening Reassessed

### The Laboratory Investigation of Congenital, Perinatal and Neonatal Infection

*Public Health Laboratory Service Working Party*

1990 ISBN 0 901144 27 4 Paperback 148x210mm 68pp £6.50

TORCH screening is widely requested in the investigation of infants and pregnant women for congenital as well as perinatal and neonatal infections. However, there is concern among microbiologists that such requests are often inappropriate and that resources would be better utilised if requests were targeted more specifically. There is also the intrauterine transmission of agents not encompassed by TORCH, such as Treponema pallidum, human immunodeficiency virus (HIV) and parvovirus B19. Has the time come to expand TORCH or to abolish it?

The book recommends that TORCH be abolished; in its place the working party provides a systematic guide to the investigations appropriate for each agent in mother, fetus, neonate and infant. Preventive screening is also discussed.

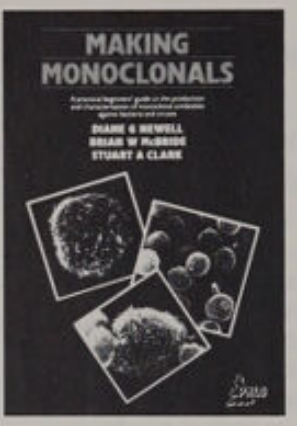
> *'... straightforward guide to the diagnosis and management of congenital, perinatal and neonatal infection.'*
>
> Abstracts on Hygiene and Communicable Diseases

## Hygiene for Hydrotherapy Pools

*Public Health Laboratory Service Working Group*

1990 ISBN 0 901144 26 6 Paperback 148x210mm 31pp £5

This booklet offers helpful and practical advice on the planning, design and management of hydrotherapy pools, covering patient safety, general hygiene and pool water quality. The PHLS-convened working group included members of the Central Sterilising Club, a physiotherapist with experience of hydrotherapy management, a water treatment chemist, an environmental health officer and five members of the PHLS Microbiology of Water Committee. Together they have produced a valuable guide for physiotherapists and others concerned with installing and running hydrotherapy pools.




## Making Monoclonals

A Practical Beginners' Guide to the Production and Characterisation of Monoclonal Antibodies against Bacteria and Viruses

***Diane G Newell, Brian W McBride, Stuart A Clark***
*PHLS Centre for Applied Microbiology and Research*

1988 ISBN 0 901144 23 1 Paperback 148x210mm 94pp £10

This book is a practical guide for first-time monoclonal makers. It contains all the information needed to choose and prepare the antigens, immunise the animals, undertake a fusion, characterise the supernatants and purify the selected monoclonal antibodies.

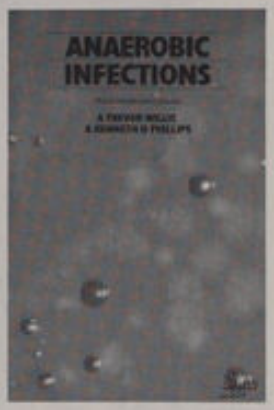
With this information, even laboratories with only modest tissue culture facilities can make monoclonals successfully - and thus provide a springboard for the production (and possibly sale) of monoclonal antibodies for diagnosis.

> *'... all you need to know about raising monoclonal antibodies. Its technical detail is thorough, precise and well written .'*
>
> Medical Laboratory Science

> *'Every aspect of antibody production is covered and the authors are to be complimented on their thorough approach to the subject.'*
>
> Journal of Clinical Pathology

## Anaerobic Infections
Clinical and Laboratory Practice

***A Trevor Willis***,
*Director, Luton Public Health Laboratory*
***Kenneth D Phillips***,
*formerly Principal Microbiologist,*
*PHLS Anaerobe Reference Unit*



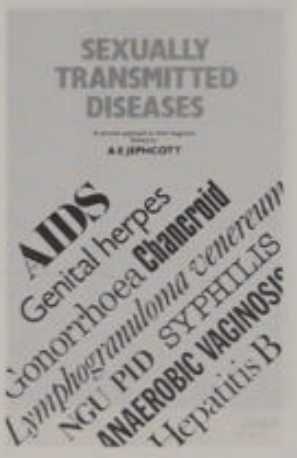

1988 ISBN 0 901144 22 3 Paperback
138x216mm 169pp £20

Anaerobic bacteria cause a variety of disease processes in man - including wound and surgical infections such as tetanus and gas gangrene, botulism and *Clostridium perfringens* food poisoning, and non-clostridial infections like abscess, bacteraemia and empyema.

This book reflects developments in clinical anaerobic bacteriology and taxonomy. The chapters on methodology emphasise accuracy and convenience for day-to-day use in a busy acute clinical laboratory. There are nine chapters, 30 tables, 23 figures and over 350 references.

> *'This ... beautifully written book ... is packed with clinical information and useful details of practical laboratory methods. ... an essential reference for both clinical microbiologists and medical laboratory scientific officers.'*
>
> Journal of Infection

> *'... much helpful and practical advice for the routine laboratory. The high level of interest among medical microbiologists should ensure this book finds a place on the shelf of every diagnostic laboratory.'*
>
> Journal of Hospital Infection




## Sexually Transmitted Diseases

A Rational Approach to their Diagnosis

*Edited by*
***A E Jephcott**, Director,*
*Bristol Public Health Laboratory*

1987 ISBN 0 901144 21 5 Paperback
145x230mm 87pp £10

We have seen an explosion in the incidence of some sexually transmitted diseases, a degree of containment in others; dramatically, the appearance of a new one - AIDS. But however the pendulum swings, the necessity for good laboratory support remains.

This book considers sexually transmitted diseases as a group, as is often convenient, and is a practical guide to current techniques. The rational approach of its seven chapters gives laboratories the information necessary to select and perform the appropriate tests and interpret results.

## Public Health Virology

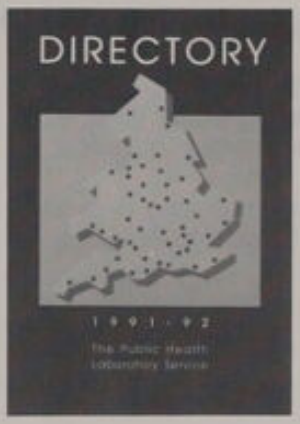
12 Reports

*Edited by **Philip P Mortimer**, Director, Virus Reference Laboratory, Central Public Health Laboratory*

1986 ISBN 0 901144 20 7 Paperback 150x225mm 194pp £15

Viruses are the cause of most transmissible disease in the community, and developments in their diagnosis, surveillance and control are a matter of keen interest to microbiologists and others concerned with the public health. This book, which was followed in 1991 by *Current Topics in Clinical Virology*, is a collection of essays on some of the important work in medical virology undertaken by public health virologists.

## PHLS Directory




ISSN 0141-9692
Paperback A5 approx 140pp

Updated annually. Price of current edition listed on order form.

This comprehensive Directory contains details of staff who work within the PHLS and of the services provided. It lists the members of the PHLS Board and its establishments - Headquarters, the Central Public Health Laboratory, the Communicable Disease Surveillance Centre, the Centre for Applied Microbiology and Research, and the 52 area and regional laboratories. Information is provided on microbiological reference facilities and the availability within the PHLS or elsewhere of certain vaccines or other materials. A separate section lists PHLS committees and working parties. Indexed.

- Staff
- Locations
- Reference facilities
- Vaccines etc
- Committees
- Index

## PHLS Microbiology Digest

ISSN 0265-3400
Quarterly, approx 150
pages per volume (year).

See order form for
subscription details.

The *Microbiology Digest* is written mainly by specialists within the PHLS and is aimed at the broadest professional audience. It contains selected articles on current infections and laboratory techniques alongside regular features on the activities of the PHLS, conference news and a special section on computing in microbiology. Recent issues have included articles on:

- Listeria in food
- Laboratory diagnosis of *Escherichia coli* 0157 infections
- Antibiotic therapy in *Escherichia coli* 0157 infections
- Multiresistant *Salmonella typhi* in the UK
- Laboratory diagnosis of *Chlamydia trachomatis*
- HIV seroepidemiology in the USA
- Public health aspects of cyanobacteria (blue-green algae)
- Water microbiology and privatisation
- Implications of 1992 EC regulations for microbiology and public health
- The UK National External Quality Assessment Scheme for Microbiology
- Computing in epidemiology and microbiology

## PHLS Library Bulletin

ISSN 0267-6850
Weekly, over 18,000 references a year.

See order form for subscription details.

This current awareness bulletin is aimed specifically at those concerned with public health and medical microbiology. Its scope is diagnosis and control of communicable disease - including infection control, food poisoning, water and airborne infections and immunisation. Each week it lists some 350 references chosen from 300 core biomedical journals. All references are selected from journals received in the PHLS libraries and therefore are easily available. More than 95% are in the English language. A list of the journals scanned is published annually.

Quarterly supplements widen coverage further. These list papers from journals not held by the PHLS Central Library, which have been acquired for its Reprint Collection.

An invaluable resource in the field, the *Library Bulletin* has a long established reputation for relevance and timeliness. New developments include an improved format for ease of use, and six-monthly author and subject indexes.

## PHLS HIV Bulletin

ISSN 0958-1316
Monthly, over 2,500 references a year.

See order form for subscription details.

The *HIV Bulletin* is a subset of citations contained in the *Library Bulletin*. It has been developed especially for those concerned with the laboratory diagnosis of HIV and the transmission of AIDS, rather than with the clinical aspects of AIDS. It consists of monthly listings, mainly articles from the 300 core biomedical journals, 95% in the English language, which are scanned regularly. The emphasis is on journals which are easily obtainable. Articles from other journals are included on an *ad hoc* basis. In addition, relevant books, conference proceedings and reports are listed.

The *HIV Bulletin* is arranged under broad subject areas - for example, transmission, infection control, testing, health education. Key words, reflecting the main theme of each article, are listed after each citation. Each issue carries a list of the journals appearing in it. Author and subject indexes are published at the end of June and December, and a list of all the journals regularly scanned is published annually.

Existing subscribers to the *Library Bulletin* whose principal interests are AIDS and HIV may find the *HIV Bulletin* an even more efficient means of keeping up to date.

The Public Health Laboratory Service is a unique national network designed to provide effective and efficient support for the diagnosis, prevention and control of infections and communicable diseases. Its 52 area and regional laboratories are spread across England and Wales and linked to epidemiological experts and specialist microbiological reference units.

At the heart of this network is the PHLS Headquarters complex at Colindale, north London, which is also where the PHLS Central Public Health Laboratory and Communicable Disease Surveillance Centre are located. The Central Public Health Laboratory provides specialised microbiological advice and is Britain's major reference centre for medical microbiology. The main role of the Communicable Disease Surveillance Centre is the national surveillance of infections and communicable diseases. The information provided by this network enables the PHLS to keep track of what infections are appearing in the country and where, and to assist in developing strategies to combat them.

PHLS publications are a valuable source of practical information and expertise for medical microbiologists, consultants in communicable disease control, environmental health professionals and others concerned with the diagnosis, prevention and control of infections and communicable diseases.


PHLS Publications 61 Colindale Avenue London NW9 5DF UK

# *The United Kingdom National External Quality Assessment Scheme for Medical Microbiology*

Pathology laboratory professionals in both public and private sectors are finding that quality issues are becoming increasingly prominent in today's competitive environment. With the formal accreditation of laboratories becoming more widespread, and with users of diagnostic services having a greater choice in the provider market, only those units demonstrating a responsible approach to quality assurance will flourish. One way of ensuring the accuracy of laboratory reports is participation in an external quality assessment scheme. By examining simulated clinical samples of known but undisclosed content, the laboratory manager is able to monitor the effectiveness of routine internal quality control procedures.

## *The nature of the Scheme*

The UK National External Quality Assessment Scheme for microbiology is administered by the Public Health Laboratory Service (PHLS), a nationwide network of microbiology laboratories which has accumulated a wealth of experience in the detection, prevention and control of human infections. The Scheme, which has been in operation since 1971, conforms to World Health Organisation recommendations on external quality assessment. Being the most comprehensive of such schemes in Europe it attracts a growing number of participants, currently 656, from the public, private and commercial manufacturing sectors of the UK as well as of 25 countries throughout the world. The Quality Assurance Laboratory of the PHLS Central Public Health Laboratory is exclusively devoted to the operation of the Scheme, whose staff are supported and advised by a wide range of experts in all disciplines of clinical microbiology.

## *Types of specimen*

An extensive range of 15 distribution types is offered, covering most areas of testing within bacteriology, mycology, parasitology, and virology. Each distribution type contains the full spectrum of specimens likely to be encountered in the laboratory, including specimens from patients having travelled overseas. Many participants find the educational aspects of the Scheme most useful, as they encounter organisms that may not arise

frequently in their own laboratories. Any participant failing with a specimen may request a repeat specimen free of charge. The reliability of specimens is ensured by rigorous quality control procedures in the Quality Assurance Laboratory.

## *Participation*

The Scheme is open to all diagnostic microbiology laboratories and manufacturers of reagents. Participants can choose the types of specimen appropriate to their laboratory. Registration is for a minimum of one full year, for which participants are required to pay a fee in advance. Annual charges and numbers of specimens vary for each distribution type, and full details may be obtained from the Quality Assurance Laboratory.

## *Assessment of performance and feedback*

Participants are allocated numerical scores for their reports, based on their level of correctness: the scores of all participants are collated and analysed; each participant receives an individual computer print-out detailing their performance and comparing it with that of laboratories examining the same specimens. A distribution summary is also provided, giving details of overall performance, methods used, and educational comment where participants have experienced difficulty. Annual record sheets and reports are also made available.

NEQAS

**Please send me further information on the UK NEQAS for Medical Microbiology**

Name _____ Address _____

Department _____

Organisation _____

**Please return to: Quality Assurance Laboratory, PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, United Kingdom**

## *Confidentiality*

Each laboratory participating is allocated a unique identification number. All matters of scoring and communication of results are dealt with under this code number in the strictest confidence.

## *Future developments*

The Scheme maintains a programme of expansion to cater for the existing and developing needs of diagnostic microbiology. Proposed new distribution types will cover the culture of mycobacteria, the detection of anti-HBs, anti-HCV, anti-CMV and anti-HAV.

## *Further details*

Those who would like to obtain further information are invited to complete and return the attached enquiry slip.

Enquiries by telephone can be
made to the Scheme Organiser at
the Quality Assurance Laboratory
on 081-200 4400 ext. 3913.

Public Health Laboratory Service
61 Colindale Avenue, London NW9 5DF
United Kingdom

# The PHLS Food Microbiology External Quality Assessment Scheme

## The Public Health Laboratory Service (PHLS)

The PHLS is a network of 52 microbiological laboratories strategically distributed throughout England and Wales, which are linked to central specialist microbiological reference and epidemiological facilities. Its remit is the diagnosis, prevention and control of human infections and communicable diseases. As part of this responsibility, all 52 laboratories provide full clinical diagnostic services for food-borne infections, as well as the testing of foods, milks, waters and other environmental specimens. Through this work, the PHLS has built up over the years an expert reputation for its public health and food microbiology.

## Please send me further information on the PHLS Food Microbiology External Quality Assessment Scheme

I would be interested in:

- ☐ Pathogens (eg salmonellas, listeria)
- ☐ Indicator organisms (eg coliforms, faecal streptococci)
- ☐ Spoilage organisms
- ☐ Yeasts and moulds
- ☐ Quantitative estimation of organisms

Other, please specify: _____

Name _____

Organisation _____

Position _____

Address _____

_____

_____

**Please enclose an example of your organisation's letter-headed paper.**

## Confidentiality

Analysis of results will be communicated by the PHLS to each participating laboratory in strict confidence and will not be communicated to third parties. Confidential consultant and technical advice will also be available from the PHLS for those participating laboratories who wish to discuss their results or receive repeat specimens free of charge.

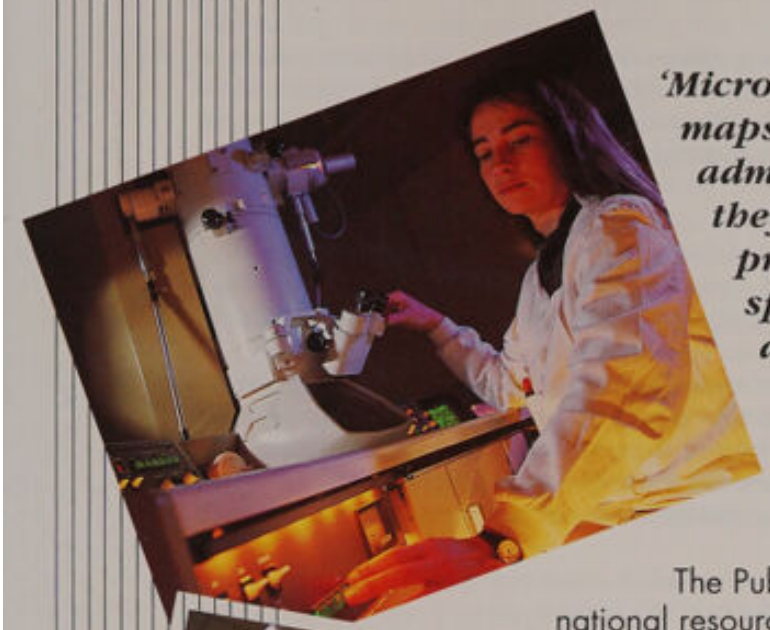
## Further information

If you or your organisation are interested in receiving further details of this scheme, please complete and return the attached form. It would be of help in further development of the scheme if you could also complete the section indicating the type of samples that would be of particular relevance to your work.

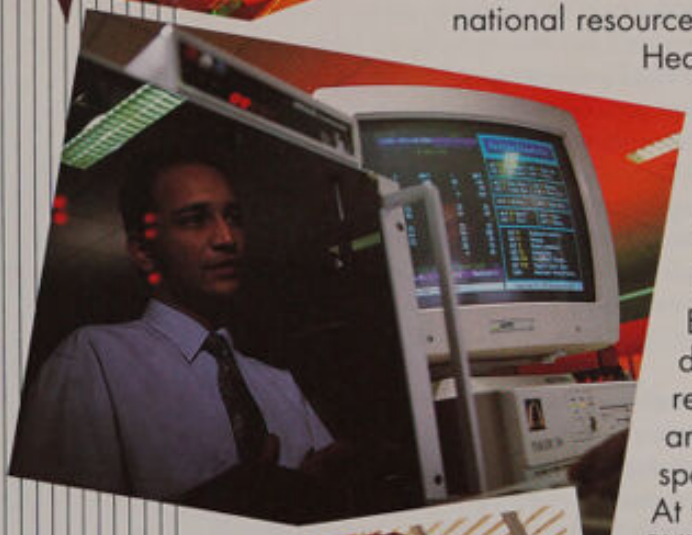
TO: The PHLS Food Microbiology External
Quality Assessment Scheme
Food Hygiene Laboratory
PHLS Central Public Health Laboratory
61 Colindale Avenue
LONDON NW9 5HT

# NETWORK PHLS

*The role of the Public Health Laboratory Service*



***'Micro-organisms do not understand maps, they do not understand administrative boundaries. Where they find people they will cause problems. Communicable diseases spread from one person to another – and if you stop for a moment the micro-organisms will have stolen a march on you...'***



The Public Health Laboratory Service is a unique national resource working closely with the National Health Service and funded principally by the Department of Health and the Welsh Office. Our major objective is to provide the most effective and efficient service possible to support the diagnosis, prevention and control of infections and communicable diseases in England and Wales. Our first line of defence in this work are our 52 area and regional Public Health Laboratories; these are linked to epidemiological experts and specialist microbiological reference units. At the heart of the organisation is the PHLS Headquarters complex at Colindale in North London, which is also where our Central Public Health Laboratory (CPHL) and our Communicable Disease Surveillance Centre (CDSC) are located. Every day this network examines over 25,000 human clinical specimens and environmental samples. The constant flow of microbiological data from this work is fed through the network for central analysis. This places the PHLS in the unique position of being able to keep track of what infections are appearing in the country, and where. Armed with this information we can develop strategies to assist in keeping infectious micro-organisms in check.

PHLS

## The fight against infection

***'We are either trying to find out what is causing illness in a patient in hospital or we are trying to find out what is causing outbreaks and illness in the community, and we want to go right back to the very basic cause of the problem. Just identifying the problem is not enough, particularly with outbreaks in the community. We have to go and see what has gone wrong...'***

We don't just map out infections in order to detect widespread problems, we are also deeply involved in individual patient care. Each of our 52 area and regional laboratories serve the NHS by undertaking for them a substantial part of their diagnostic clinical microbiology. If more detailed investigations are needed, we have specialist reference units where these can be undertaken. We help identify infections in often seriously ill patients, and advise their clinicians about the right treatment to combat them. We also offer advice on the best tests to run to help diagnose the patient's condition. But our role doesn't end there; we want to stop more people becoming ill. That might mean simply offering preventive treatment to family contacts of the infected person, or it might mean helping to track down the source of an infection in the community or the hospital to stop it spreading.

## Detective work: identifying disease

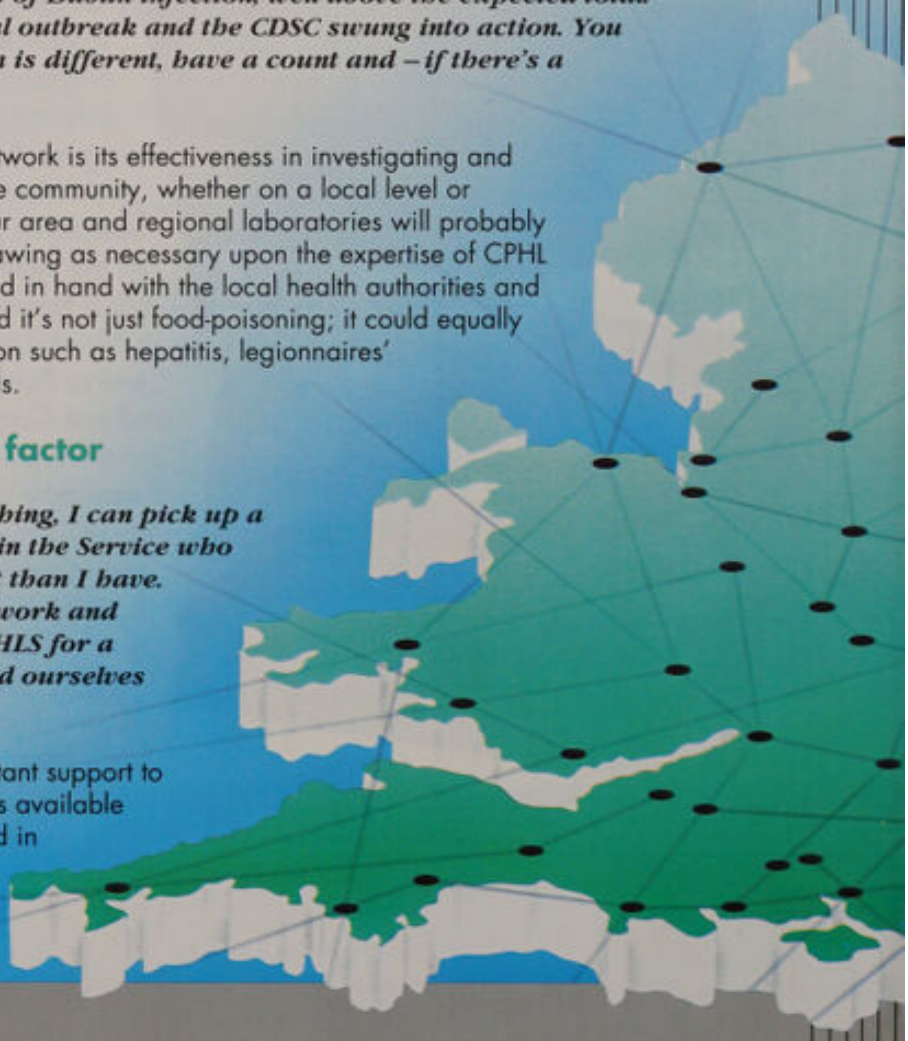
***'Chester had the first household outbreak. From a sample of an unpasteurised soft cheese eaten at a dinner party they pulled out a salmonella and sent it to our reference laboratory here at CPHL for sub-typing. It turned out to be a serotype called Dublin, a highly invasive form of salmonella. Then we had a similar household outbreak identified by Hereford, again with the clue of an unusual cheese. During the first week of December we identified 22 cases of Dublin infection, well above the expected total. On 7 December I notified a national outbreak and the CDSC swung into action. You spot the start, spot that the pattern is different, have a count and – if there's a human threat – act!'***

An outstanding feature of the PHLS network is its effectiveness in investigating and controlling outbreaks of infection in the community, whether on a local level or nationally. At any one time each of our area and regional laboratories will probably be dealing with several outbreaks, drawing as necessary upon the expertise of CPHL and CDSC – and always working hand in hand with the local health authorities and environmental health departments. And it's not just food-poisoning; it could equally well be an outbreak of another infection such as hepatitis, legionnaires' disease, meningitis or cryptosporidiosis.

## The detectives: the human factor

***'If I'm having difficulty with something, I can pick up a telephone and speak to somebody in the Service who probably has more knowledge of it than I have. The PHLS exists because of its network and you've only got to be around the PHLS for a short time to realise that we regard ourselves as members of a large family...'***

Each individual laboratory has the instant support to hand of all the expertise and resources available to the PHLS. Such back-up can be used in solving local problems, whether in helping to formulate advice to



deal with an infection control difficulty in a hospital operating theatre or in enlisting specialist staff to help in an outbreak investigation. We have some three thousand medical experts, scientists, medical laboratory scientific officers, ancillary workers and administrative staff working within the PHLS. A constant exchange of information goes on between them – through weekly laboratory reports, through our computerised epidemiological information system, through meetings and working groups, or simply through informal contact – 'picking up the telephone'. Their input – coming from all quarters of the PHLS – is what distinguishes us as a laboratory network, allowing us to bring our combined information and expertise to bear in identifying human infections and preventing their spread.

## Prevention: stopping it before it starts

***'Last week there was a major public event in my patch where for the third year running we took two hundred samples of food and did full microbiology. With the local environmental health people we then talked to the caterers and said that an improvement in quality was required. What we are trying to do is to be proactive rather than reactive. We have not waited until there has been an outbreak...'***

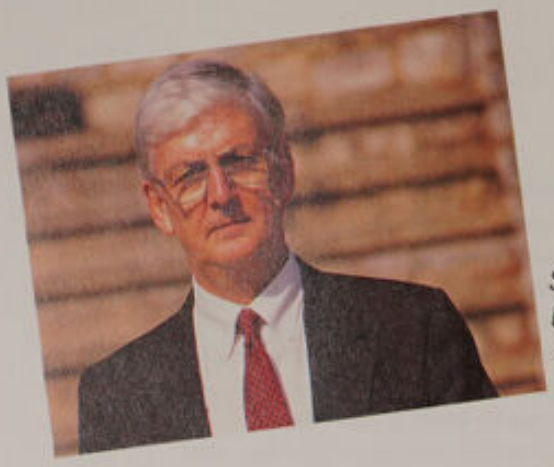
One aspect of our work that rarely makes news is our role in disease prevention. PHLS working parties are always in action looking into particular problems of infection and recommending control measures, often in alliance with the Department of Health and other bodies. We have also organised special surveillance groups ('PHLS chains') where a number of our laboratories operate together to do regular national surveys on, for example, the microbiological quality of food and water. We are also involved on a more local level, looking into potential trouble spots. These could include working together with district health authority engineers to ensure that hospital water systems are not maintained and operated in such a way to permit the bacterium that causes legionnaires' disease to grow and be dispersed, or collaborating with a local authority's Environmental Health Department in improving food hygiene standards.

## Collaboration: the sharing of information and expertise

***'Since 1982 clinicians in this country have been encouraged to report in confidence cases of AIDS to the PHLS; this was augmented in 1985 by the confidential reporting by microbiologists of positive test results for infection with the human immunodeficiency virus (HIV) which causes AIDS. This collaboration has given us extensive information on how the AIDS epidemic is developing in this country; information that has been fed back to help plan for hospital and treatment needs and to develop educational and preventive programmes...'***

We thrive on collaboration – within the Service and outside it. Information we need to map out communicable diseases comes in to us not only from our own laboratories, but from many other sources – other NHS laboratories, Consultants in Communicable Disease Control, Environmental Health Departments, the Office of Population Censuses and Surveys, and GP 'spotter practices'. The information flow is two-way, though; the understanding we gain from our work is available as advice at many levels – to local and central government, to the hospital service, to general practitioners and to private companies.

We also maintain links with a great many national and international organisations involved in health and communicable disease. These include bodies such as the Blood Transfusion Service, the Medical Research Council, universities and medical schools in the UK - and, at the international level, epidemiological centres or public health organisations like the Centers for Disease Control in Atlanta, USA, and the World Health Organisation.



*Sir Joseph Smith, Director of the Service*

## LINKS IN THE NETWORK

### Area and regional public health laboratories

All the 52 area and regional PHLS laboratories are either located within or linked to district general hospitals, for whom they carry out diagnostic clinical microbiological services. They also provide investigative services relating to public health in their locality, working in close collaboration with NHS Consultants in Communicable Disease Control and Environmental Health Officers, local GPs and others involved in community care.

### The PHLS Headquarters Office

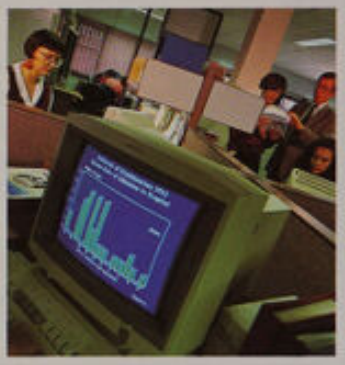
The HQ Office at Colindale acts as the centre for the PHLS. It houses Business Planning, Finance, Personnel, Estates, Supplies and Computing Departments and also includes the Co-ordination Unit which helps ensure that the optimum response and support are provided to resolve local hospital, local community microbiological and epidemiological and national infection problems.

### The Central Public Health Laboratory


The CPHL is a complex of laboratories that together make up the Service's main reference and specialist microbiological advice centre. As well as assisting our own area and regional laboratories, the CPHL also provides services to NHS laboratories and to other appropriate health organisations. It comprises eight divisions, each of which undertakes investigations and research and development: the Division of Enteric Pathogens; the Food Hygiene Laboratory; the Division of Hospital Infection; the Division of Microbiological Reagents; the Mycological Reference Laboratory; the National Collection of Type Cultures; the Quality Assurance Laboratory; and the Virus Reference Laboratory.

### The Communicable Disease Surveillance Centre

The main role of the CDSC is the national surveillance of infections and communicable disease, working in liaison with area and regional laboratories and with specialist reference laboratories. It acts as the network's epidemiological nerve centre, collecting, analysing and disseminating data on the occurrence and spread of communicable diseases, and it provides assistance in the field with the investigation and management of outbreaks. The CDSC comprises five divisions: the Surveillance and Information Division, which produces a weekly Communicable Disease Report and operates an electronic communications and information system; the Immunisation Division; the Field Services and Training Division; the Regional Services Division; and the PHLS AIDS Centre (HIV, Sexually Transmitted Disease and Hepatitis Division).



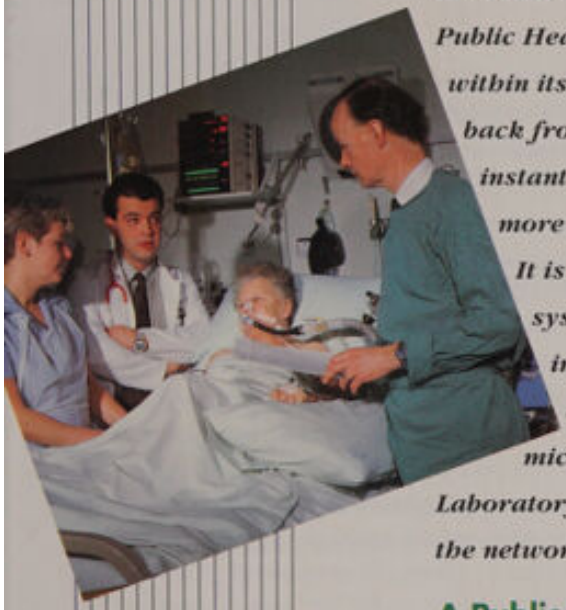
### The Centre for Applied Microbiology and Research

The CAMR is located in Wiltshire and comprises laboratories and production facilities organised into four main divisions: the Pathology Division; the Biologics Division; the Biotechnology Division; and the Central Services Division. As well as providing a number of important reference services and expertise for the PHLS, CAMR also generates income through its applied research in microbiology and biotechnology.

Prepared for the Public Health Laboratory Service by the Central Office of Information, 1990
Printed in the UK, October 1991 PUHE J0006 AR

# THE WORK OF AREA AND REGIONAL PHLS LABORATORIES

*Public Health Laboratory Service*



***'The unique benefit of a Public Health Laboratory is that it is part of the PHLS network. As part of that network, each area and regional Public Health Laboratory monitors the infectious diseases occurring within its area, reports on them centrally, and takes information back from the centre for local dissemination and advice. It has instant access to a huge range of specialist reference services for more detailed tests on micro-organisms or help in investigations. It is also linked to a central epidemiological computing system that enables it to see at a glance what infections are appearing around the country. In a way, each area and regional laboratory is a microcosm of the whole Public Health Laboratory Service – consult one and you consult the network ...'***

## A Public Health Laboratory has the following main functions:

– To provide a microbiological laboratory testing service to the highest standard for the diagnosis of diseases for hospital based clinicians, general practitioners and occupational health physicians; this covers bacteriology, virology and serology and also fungal and other infections.

– To provide a comprehensive consultant microbiological service in both clinical and public health microbiology, including hospital infection control services and advice on antibiotic policies and therapies, sterilisation and disinfection, microbiological investigation of problem cases and immunisation needs.

– To work closely with local Consultants in Communicable Disease Control and Environmental Health Officers on the prevention and control of infection, including the investigation of infectious disease outbreaks and advice on appropriate control measures.

– To undertake public health microbiology as required, for example, the testing of food, milk, water, environmental and outbreak specimens.

– To collaborate closely with PHLS Reference Laboratories and with the PHLS Communicable Disease Surveillance Centre in the investigation of infections.

– To participate in local teaching and technical training about infection control problems and their avoidance.

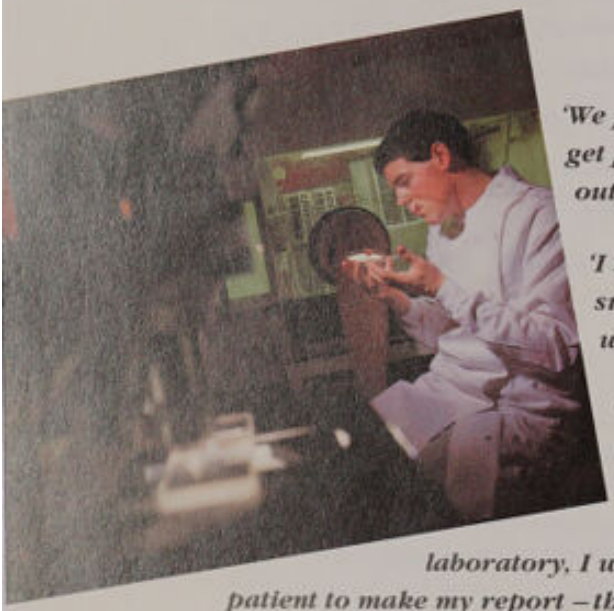
The 52 area and regional Public Health Laboratories are, with a few exceptions, situated in local hospitals. As well as

PHLS

providing a full clinical microbiology service to the hospitals in which they are based, they also undertake work for hospitals in neighbouring district health authorities. Over half of the laboratories provide services for more than five districts. Similarly each laboratory offers diagnostic testing and microbiological support and advice to general practitioners within their district health authority and to GPs in adjoining districts. Important national microbiological reference facilities are also located in some of the area and regional laboratories.

In addition to health authority work, area and regional laboratories also carry out extensive investigations for the Environmental Health Departments of local authorities. The majority of laboratories serve between five to ten local authorities.

Schools, armed forces camps, prisons, factories and ports are among the other regular users of Public Health Laboratory facilities. Increasingly, commercial companies – for example, those involved in food processing – are taking advantage of the expertise available in PHLS laboratories and joint research projects with industry are also undertaken.



## Providers of a clinical microbiology service

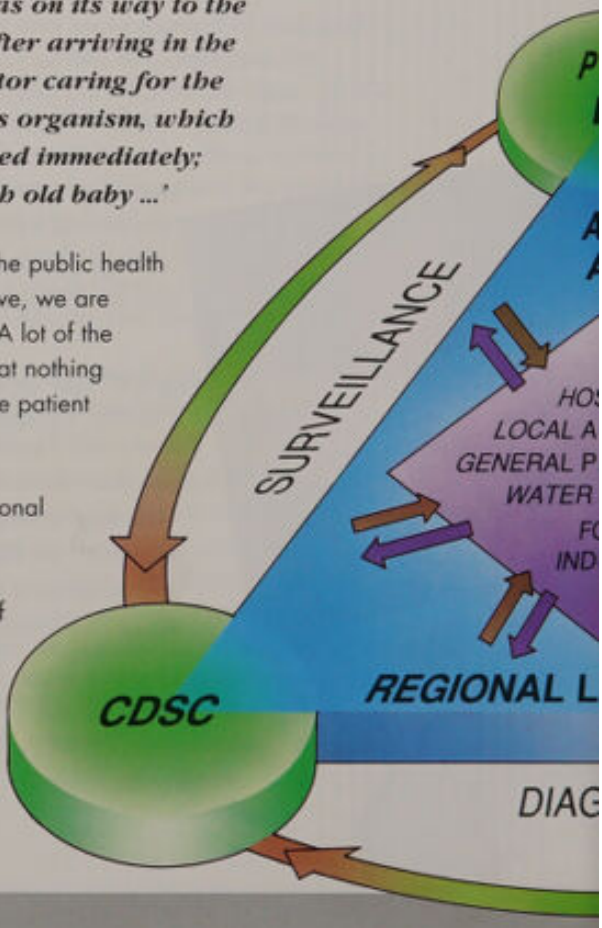
***'We provide a clinical microbiology service. We see patients, and I get patients with difficult infections referred to me. I run a small outpatient clinic as well ...***

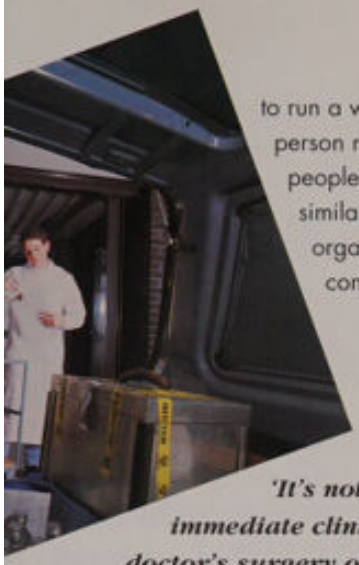
***'I talk to clinicians and I diagnose cases of infectious diseases in surgical and medical patients on a daily basis. One of us visits the ward every day ...***

***'Last week I was the Medical Laboratory Scientific Officer on call. It was one o'clock in the morning. A cerebro-spinal tap had been taken, the specimen was on its way to the laboratory. Half an hour after arriving in the laboratory, I was able to telephone the doctor caring for the patient to make my report – the finding of the haemophilus organism, which can cause severe meningitis. Treatment could then be started immediately; treatment that could well have saved the life of an 18-month old baby ...'***

Because of our name, people tend to associate PHLS laboratories with the public health aspect of our work. However, as can be seen from the staff quoted above, we are very committed to patient care. It's not all life and death investigations. A lot of the day-to-day laboratory work is exclusion – routine monitoring to check that nothing is wrong. It all goes though to help build a final picture to ensure that the patient gets the best treatment.

With new organisms and new illnesses, the work of each area and regional laboratory is becoming more extensive. The number of specimens are increasing, while the range of tests that have to be run are becoming increasingly numerous and complex. This is particularly so in the case of investigations for those infected with the human immunodeficiency virus (HIV) which causes AIDS. Opportunistic infections can occur in the HIV-infected person and lead to serious disease. There is a need therefore

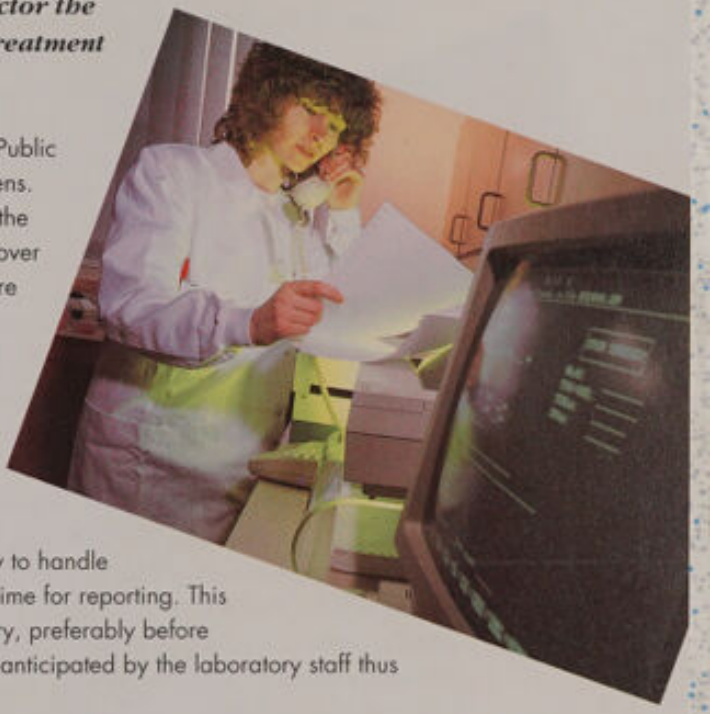




to run a wide array of laboratory investigations in such cases looking for infections that in the ordinary person might not cause a problem but do in the HIV-infected patient. There are other examples of people whose immune system is impaired – for example, those with bone marrow transplants – who similarly require extensive, detailed and rapid investigations because for them normally innocuous organisms can be life-threatening. In recent years, Public Health Laboratories have built up considerable expertise in such investigations.

## The personal touch

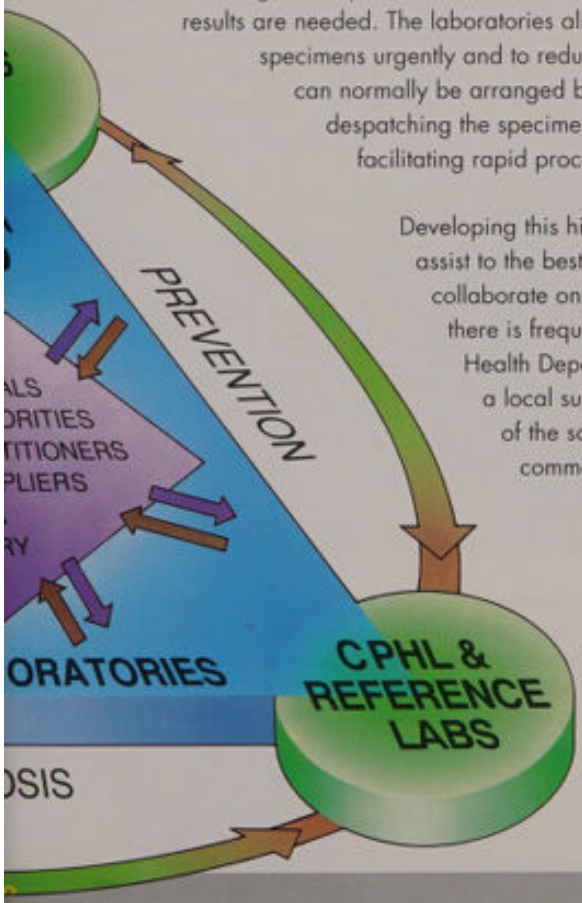
***'It's not feasible to telephone all results, but any result that we consider to be of immediate clinical importance will be telephoned through to the doctor's surgery or to a houseman. That also gives the doctor the opportunity of discussing with us the most appropriate treatment or whether any further investigations are needed ...'***



Obviously in containing infectious diseases, speed is vitally important. Public Health Laboratories pride themselves on their response time on specimens. However, it's not just getting the laboratory work done, it's also letting the right person know the result quickly. Personal contact either directly or over the telephone to confirm a positive result is given a high priority. We are also developing a computerised laboratory reporting system that will enable those submitting specimens for analysis to receive their reports directly by computer themselves. Combined with continued direct access to specialist advice from our laboratories, this development will enhance still further the service we can provide.

Even with a good response time, there are instances when very rapid results are needed. The laboratories also offer therefore the facility to handle specimens urgently and to reduce the normal turn around time for reporting. This can normally be arranged by telephoning the laboratory, preferably before despatching the specimen, so that its arrival can be anticipated by the laboratory staff thus facilitating rapid processing and reporting.

Developing this high level of rapport with our regular users enables the laboratories to assist to the best effect in an urgent situation, and also provides the contact necessary to collaborate on proactive work – prevention is always better than cure. In particular there is frequent interaction between the laboratories and their local Environmental Health Departments. For example, a laboratory might work with them in organising a local survey of specific foods, or following identification of a number of cases of the same type might set up a joint study to investigate whether there is a common problem.

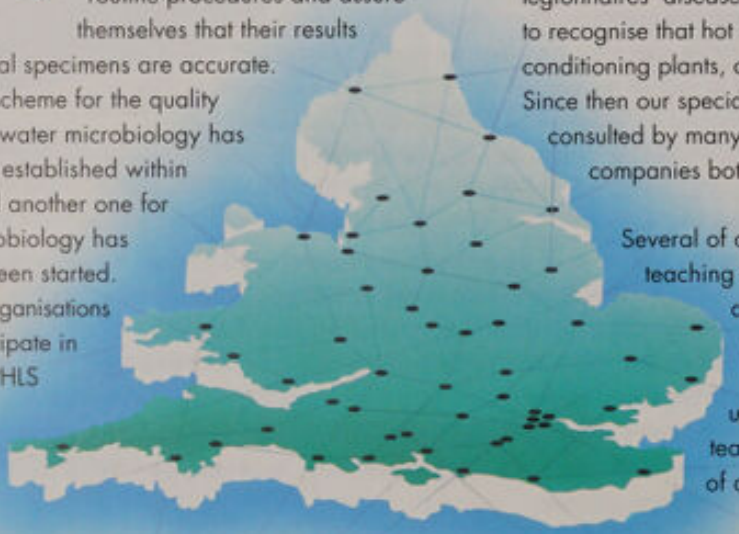


## Quality assurance

***'This is carried out by periodic internal audit of specimen throughput and also by regular discussion with general practitioners and consultants about appropriateness and timeliness of tests and reports. All reports are validated and authorised ...'***

Quality assurance to establish the accuracy of testing is taken very seriously by PHLS area and regional laboratories. As well as regular internal scrutiny of methods, all laboratories take part in the UK National External Quality Assessment Scheme for Microbiology (NEQAS). This scheme is administered at the PHLS Central Public Health Laboratory, but PHLS area and regional laboratories participate in exactly the same way as other laboratories from the NHS, private health care sector, the armed forces and overseas. By participating in NEQAS all our laboratories can monitor the efficiency of their routine procedures and assure themselves that their results with clinical specimens are accurate.

A further scheme for the quality control of water microbiology has also been established within PHLS, and another one for food microbiology has recently been started. Outside organisations can participate in all these PHLS quality assurance schemes.



## Research and development

***'You need people who are not only looking at an individual patient, but who are also looking at where the organism came from, and the sort of factors that have enabled that micro-organism to get into a situation where it can cause infection ...'***

Uniquely our area and regional laboratories are not only diagnosing on a daily basis thousands of human infections, but are also monitoring the appearance of causative organisms in the environment, in hospitals, in animals, in foodstuffs. Micro-organisms are constantly changing and adapting, acquiring resistance to new antibiotics, exploiting new situations favourable to their growth and distribution. Infectious diseases can also be introduced from abroad. The PHLS network would be among the first to spot such changes. Our network has always played a prominent role in research on new infectious problems. Our close surveillance of legionnaires' disease meant that this country was the first to recognise that hot water systems, as well as air-conditioning plants, could be the source of this infection. Since then our specialists in this field have been consulted by many organisations and commercial companies both in this country and abroad.

Several of our laboratories are located in teaching hospitals or associated with academic departments of microbiology. Staff in these laboratories undertake undergraduate or postgraduate teaching and collaborative research of a high standard.

***At a local level the range and depth of PHLS specialist knowledge is available by consulting your Public Health Laboratory. Design of hospitals, and particularly operating theatres, changes in sterilisation equipment, food processing techniques or water treatment can all have considerable impact on human infectious disease. The PHLS has active research programmes in all these fields – and many more. Our laboratories always welcome the opportunity of sharing and increasing their expertise through collaborative ventures with either the public or private sector.***

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# News Release

92/12 26 October 1992

## QUARTERLY AIDS AND HIV FIGURES

The Public Health Laboratory Service today published in the Communicable Disease Report AIDS and HIV figures for the United Kingdom. These show that in September there were 127 new reports of AIDS cases bringing the cumulative total to 6,555 cases.

The cumulative total of recognised HIV infections is now 18,526. New reports of HIV infection may relate to people who have recently become infected, to people infected some time before choosing to be tested, or to people first tested when they develop AIDS.

**Notes to Editors:**

1. Epidemiological surveillance of the Acquired Immune Deficiency Syndrome (AIDS) was begun at the PHLS AIDS Centre in collaboration with the Communicable Diseases (Scotland) Unit (CD(S)U) in 1982. Surveillance of Human Immunodeficiency Virus (HIV) infection, the cause of AIDS, commenced in late 1984.

2. PHLS and CD(S)U work in collaboration with colleagues in the Oxford Haemophilia Centre (coordinating national information on haemophiliacs) and with colleagues in the Department of Paediatric Epidemiology, Institute of Child Health.

3. AIDS case reports are compiled from voluntary confidential reports by clinicians directly to PHLS and CD(S)U. A summary report is released monthly.

4. HIV infection reports are made through a voluntary confidential reporting by the microbiologists in laboratories where HIV tests are carried out. They are summarised and released with the monthly AIDS figures at the end of each quarter.

5. Due to the length and variability of the period between infection with HIV and development of AIDS (average of between 8-10 years), reports of AIDS cases do not give a picture of the current spread of HIV infection.

6. Information on deaths is based on reports to PHLS and CD(S)U from clinicians, supplemented by information on death entries obtained from the Office of Population Censuses and Surveys and the Registrar General for Scotland.

7. There are many people currently being treated for disease related to HIV infection who do not yet fulfil the surveillance case definition for AIDS.

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Further data on AIDS is available in the PHLS Communicable Disease Report, which can be subscribed to by contacting Editor CDR, PHLS Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ.

# AIDS and HIV-1 infection in the United Kingdom: monthly report

During **September 1992**, 127 new cases of AIDS were reported. Ninety-three were probably infected through sexual intercourse between men (14 died), fifteen through sexual intercourse between men and women (2 died), seven through injecting drug use, five through either injecting drug use or sexual intercourse between men (3 died), and five through blood factor treatment. The exposure categories of two cases were undetermined.

Since reporting began in 1982, a total of 6555 AIDS cases (6130 male and 425 female) has been reported (Table 1), of whom 4061 (62%) are known to have died. Four hundred and seventeen AIDS cases (70 deaths) were reported in the third quarter of 1992. This is the largest number reported in any quarter to date. The previous highest quarterly total, which was 391, occurred in the fourth quarter of 1991.

The number of male AIDS cases increased by 17% (from 1170 to 1367) in the two twelve month periods from October 1990 to September 1992; female cases increased by 28% (from 109 to 139). The number of AIDS cases due to sexual intercourse between men and women increased by 47% (from 147 to 216), including a 39% rise (from 122 to 170) in infections probably acquired abroad, and an increase (from 12 to 25) in infections acquired in the UK not known to be from a 'high risk' partner.

Since reporting began in 1984, a total of 18,526 reports of HIV-1 infected persons has been received (Table 2). Of this total, 11,165 (60%) were infected through sexual intercourse between men. The number of persons probably infected through sex between men and women abroad increased by 38% (from 346 to 478) in the two recent twelve month periods. A similar increase was seen in the number of women infected through sex with a 'high risk' male partner, though the numbers involved were much smaller (from 35 to 48).

In the twelve month period to September 1992, heterosexual men and women accounted for 24% of AIDS cases (Table 3), and 38% of HIV-1 infections reported (Table 4).

Two-thirds of all AIDS cases continue to be reported from three of the four Thames regions - NW, NE, and SE Thames (Table 5).

The proportion of reported HIV-1 infections in each English health region that were due to sex between men and women ranged from 8% (457 of 5481 reports) in NW Thames, to 25% (167 of 662) in SW Thames (Table 6). The proportion due to injecting drug use ranged from 6% (34 of 612) in W Midlands, to 20% (52 of 265) in E Anglia.

## Paediatric data

A total of 94 cases of AIDS was reported by the end of July 1992 in children 14 years or under, 43 of whom are known to have died (Table 7). Sixty-four (68%) were infected perinatally, and 30 (32%) through contaminated blood or blood factor treatment. Forty-seven of the 403 HIV-1 infected children are known to have died (Table 8).

**Table 1 AIDS cases and deaths by exposure category and date of report: United Kingdom to 30 September 1992**

| How persons probably acquired the virus | Oct 90 - Sept 91 | | Oct 91 - Sept 92 | | Jan 82 - Sept 92 | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Deaths | Female | Deaths |
| Sexual intercourse | | | | | | | | |
| between men | 917 | - | 1066 | - | 4990 | 3141 | - | - |
| between men and women | | | | | | | | |
| "high risk" partner[1] | 5 | 7 | 8 | 12 | 22 | 10 | 42 | 23 |
| other partner abroad[2] | 75 | 47 | 103 | 67 | 307 | 164 | 169 | 64 |
| other partner UK | 7 | 5 | 14 | 11 | 31 | 13 | 26 | 14 |
| under investigation | 1 | - | 1 | - | 3 | 3 | - | - |
| Injecting drug use (IDU) | 56 | 31 | 50 | 25 | 210 | 119 | 87 | 52 |
| IDU & sexual intercourse between men | 22 | - | 23 | - | 106 | 72 | - | - |
| Blood | | | | | | | | |
| blood factor (e.g. for haemophilia) | 58 | 1 | 57 | 1 | 323 | 249 | 5 | 3 |
| blood/tissue transfer (e.g. transfusion) | | | | | | | | |
| abroad | 1 | 2 | 1 | 4 | 14 | 10 | 29 | 18 |
| UK | 1 | 4 | 2 | 2 | 18 | 13 | 18 | 12 |
| Mother to infant | 8 | 11 | 13 | 10 | 30 | 12 | 37 | 18 |
| Other/undetermined | 19 | 1 | 29 | 7 | 76 | 46 | 12 | 5 |
| Total | 1170 | 109 | 1367 | 139 | 6130 | 3852 | 425 | 209 |

1. Men and women who had sex with injecting drug users, or with those infected through blood factor treatment or blood transfusion, and women who had sex with bisexual men.

2. Includes persons without other identified risks from, or who have lived in, countries where the major route of HIV-1 transmission is through sexual intercourse between men and women.

**Table 2 HIV1 infected persons by exposure category and date of report: United Kingdom to 30 September 1992**

| How persons probably acquired the virus | Oct 90 - Sept 91 | | | Oct 91 - Sept 92 | | | Cumulative to September 1992 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | NS[4] | Male | Female | NS[4] | Male | Female | NS[4] |
| Sexual intercourse | | | | | | | | | |
| between men | 1599 | - | - | 1453 | - | - | 11165 | - | - |
| between men and women | | | | | | | | | |
| "high risk" partner[1] | 16 | 35 | - | 7 | 48 | - | 54 | 274 | - |
| other partner abroad[2] | 167 | 178 | 1 | 250 | 224 | 4 | 815 | 681 | 9 |
| other partner UK | 22 | 28 | - | 17 | 28 | - | 85 | 111 | - |
| under investigation | 32 | 30 | - | 71 | 56 | - | 157 | 158 | - |
| Injecting drug use (IDU) | 170 | 73 | - | 127 | 51 | - | 1552 | 711 | 9 |
| IDU & sexual intercourse between men | 21 | - | - | 34 | - | - | 235 | - | - |
| Blood | | | | | | | | | |
| blood factor (e.g. for haemophilia) | 41 | | - | 9 | 1 | - | 1236 | 10 | 1 |
| blood/tissue transfer (e.g. transfusion) | | | | | | | | | |
| abroad/UK | 5 | 14 | - | 13 | 10 | - | 90 | 88 | 1 |
| Mother to infant[3] | 21 | 17 | - | 15 | 11 | - | 68 | 65 | - |
| Other/undetermined | 59 | 25 | 4 | 88 | 22 | 2 | 754 | 154 | 43 |
| Total | 2153 | 400 | 5 | 2084 | 451 | 6 | 16211 | 2252 | 63 |

1 and 2. As for table 1.

3. By date of report that established infected status.
4. Sex not stated on report.

**Table 3 Sexual orientation of adult (15 years or over) AIDS cases: United Kingdom to 30 September 1992**

| Sexual orientation (regardless of exposure category) | Oct 90 - Sept 91[1] | | Oct 91 - Sept 92[1] | | Jan 82 - Sept 92 | |
|---|---|---|---|---|---|---|
| | Cases | (%) | Cases | (%) | Cases | (%) |
| Homosexual men | 794 | (65) | 940 | (65) | 4327 | (68) |
| Bisexual men | 146 | (12) | 152 | (11) | 775 | (12) |
| Heterosexual men and women[2] | 284 | (23) | 345 | (24) | 1246 | (20) |
| Total[3] | 1224 | (100) | 1437 | (100) | 6348 | (100) |

1. Period during which reports were received.
2. Includes those exposed through injecting drug use or infected through blood factor treatment or blood transfusion.
3. Excludes other/undetermined cases.

**Table 4 Sexual orientation of adult (15 years or over) HIV-1 infected persons: United Kingdom to 30 September 1992**

| Sexual orientation (regardless of exposure category) | Oct 90 - Sept 91[1] | | Oct 91 - Sept 92[1] | | Cumulative to September 1992 | |
|---|---|---|---|---|---|---|
| | Number | (%) | Number | (%) | Number | (%) |
| Homosexual men/bisexual men | 1620 | (67) | 1487 | (62) | 11400 | (66) |
| Heterosexual men and women[2] | 808 | (33) | 912 | (38) | 5775 | (34) |
| Total[3] | 2428 | (100) | 2399 | (100) | 17175 | (100) |

1. Period during which reports were received.
2. Includes those exposed through injecting drug use or infected through blood factor treatment or blood transfusion.
3. Excludes other/undetermined cases.

Tables 1–6 were prepared from voluntary confidential reports by clinicians and microbiologists sent directly to the PHLS AIDS Centre at CDSC (081 200 6868) and to the Communicable Diseases (Scotland) Unit (041 946 7120), from returns by Haemophilia Centre Directors to the Oxford Haemophilia Centre (0865 225316), and monthly returns by paediatricians to the British Paediatric Surveillance Unit.

**Table 5 Geographical distribution of AIDS cases and deaths by date of report: to 30 September 1992**

| Country or region of first report | Oct 90 - Sept 91 | | Oct 91 - Sept 92 | | Jan 82 - Sept 92 | |
|---|---|---|---|---|---|---|
| | Cases | Related deaths[1] | Cases | Related deaths[1] | Cases | Related deaths[1] |
| England: | | | | | | |
| Northern | 15 | 4 | 16 | 6 | 107 | 74 |
| Yorkshire | 33 | 18 | 36 | 14 | 167 | 114 |
| Trent | 25 | 17 | 39 | 17 | 137 | 94 |
| E Anglia | 21 | 5 | 29 | 6 | 90 | 46 |
| NW Thames | 407 | 167 | 524 | 100 | 2455 | 1481 |
| NE Thames | 279 | 120 | 360 | 61 | 1268 | 684 |
| SE Thames | 139 | 77 | 164 | 48 | 721 | 472 |
| SW Thames | 54 | 22 | 56 | 19 | 249 | 169 |
| Wessex | 29 | 12 | 42 | 15 | 155 | 105 |
| Oxford | 35 | 16 | 32 | 5 | 142 | 80 |
| S Western | 33 | 20 | 35 | 9 | 141 | 90 |
| W Midlands | 30 | 13 | 36 | 20 | 151 | 103 |
| Mersey | 21 | 13 | 10 | 6 | 79 | 60 |
| N Western | 45 | 29 | 48 | 16 | 246 | 169 |
| Wales | 20 | 17 | 15 | 5 | 92 | 69 |
| Northern Ireland | 6 | 4 | 5 | 3 | 31 | 27 |
| Scotland | 87 | 57 | 59 | 21 | 324 | 224 |
| United Kingdom total | 1279 | 611 | 1506 | 371 | 6555 | 4061 |
| Ch. Islands/Isle of Man | 1 | 1 | - | - | 5 | 4 |

1. These deaths are of patients referred to in the previous column and known to have occurred at any time up to 30 September 1992.

**Table 6 Geographical distribution and exposure category of HIV-1 infected persons: to 30 September 1992**

| Country or region of first report | Sexual intercourse | | | | Injecting[1] drug use | | | Blood | Other/ Undetermined | | | Cumulative total Oct 84 - Sept 92 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | between men | between men and women | | | | | | | | | | |
| | | M | F | NS[2] | M | F | NS[2] | | M | F | NS[2] | |
| England: | | | | | | | | | | | | |
| Northern | 177 | 26 | 21 | - | 48 | 12 | 1 | 92 | 9 | - | - | 386 |
| Yorkshire | 252 | 42 | 45 | - | 39 | 21 | - | 72 | 11 | 4 | - | 486 |
| Trent | 259 | 41 | 27 | - | 48 | 15 | - | 77 | 8 | 6 | - | 481 |
| E Anglia | 122 | 26 | 13 | - | 37 | 15 | - | 38 | 10 | 4 | - | 265 |
| NW Thames | 4238 | 202 | 255 | - | 344 | 111 | 2 | 77 | 191 | 46 | 15 | 5481 |
| NE Thames | 2180 | 258 | 283 | 5 | 180 | 99 | 2 | 197 | 236 | 39 | 21 | 3500 |
| SE Thames | 1346 | 125 | 154 | 1 | 174 | 74 | - | 141 | 72 | 26 | 1 | 2114 |
| SW Thames | 335 | 75 | 92 | - | 44 | 12 | - | 36 | 59 | 8 | 1 | 662 |
| Wessex | 243 | 18 | 23 | - | 36 | 9 | - | 42 | 11 | 6 | 2 | 390 |
| Oxford | 209 | 31 | 45 | 3 | 49 | 14 | - | 117 | 9 | 1 | 1 | 479 |
| S Western | 237 | 35 | 29 | - | 42 | 8 | - | 33 | 8 | 4 | - | 396 |
| W Midlands | 289 | 33 | 40 | - | 29 | 5 | - | 157 | 49 | 10 | - | 612 |
| Mersey | 111 | 13 | 8 | - | 11 | 9 | - | 47 | 12 | 2 | - | 213 |
| N Western | 528 | 50 | 30 | - | 54 | 27 | - | 125 | 10 | 3 | - | 827 |
| Wales | 146 | 35 | 21 | - | 11 | 2 | - | 62 | 8 | 2 | - | 287 |
| Northern Ireland | 57 | 8 | 11 | - | 2 | 3 | - | 19 | 2 | - | - | 102 |
| Scotland | 436 | 93 | 127 | - | 639 | 275 | 4 | 94 | 117 | 58 | 2 | 1845 |
| United Kingdom total | 11165 | 1111 | 1224 | 9 | 1787 | 711 | 9 | 1426 | 822 | 219 | 43 | 18526 |
| Ch. Islands/Isle of Man | 14 | 2 | 2 | - | 4 | 3 | - | 3 | 2 | - | - | 30 |

1. Includes 235 male drug users who also had sexual intercourse with other men.
2. Sex not stated on report.

**Table 7 AIDS cases and deaths in children[1] by exposure category: to 31 July 1992**

| How children probably acquired the virus | Aug 90 - Jul 91 | | Aug 91 - Jul 92 | | Jan 82 - Jul 92 | | 15 years or over at death or by 31 July 1992 | |
|---|---|---|---|---|---|---|---|---|
| | Diagnosis[2] | Report[2] | Diagnosis[2] | Report[2] | Total | Deaths | Cases | Deaths |
| Mother to infant | 16 | 20 | 18 | 22 | 64 | 28 | - | - |
| Blood | | | | | | | | |
| blood factor (e.g. for haemophilia) | 4 | 6 | 2 | 2 | 22 | 13 | 10 | 3 |
| blood/tissue transfer (e.g. transfusion) | 1 | 1 | 1 | 3 | 8 | 2 | - | - |
| Other/undetermined | - | - | - | - | - | - | - | - |
| Total | 21 | 27 | 21 | 27 | 94 | 43 | 10 | 3 |

1. All were aged 14 years or under when AIDS was first diagnosed.
2. Differences between timing of diagnosis and timing of report are due to reporting delay.

**Table 8 HIV-1 infection[1] and deaths[2] in children[3] by sex and exposure category: cumulative to 31 July 1992**

| How children probably acquired the virus | England, Wales and N. Ireland | | | Scotland | | | Total | Deaths | 15 years or over at death or by 31 July 1992 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | NS | Male | Female | NS | | | Infections | Deaths |
| Mother to infant | 59 | 54 | - | 9 | 11 | - | 133 | 29 | - | - |
| Blood | | | | | | | | | | |
| blood factor (e.g. for haemophilia) | 221 | - | - | 21 | - | - | 242 | 17 | 182 | 7 |
| blood/tissue transfer (e.g. transfusion) | 9 | 12 | 1 | 2 | 1 | - | 25 | 1 | 5 | - |
| Other/undetermined | 2 | - | - | 1 | - | - | 3 | - | 3 | - |
| Total | 291 | 66 | 1 | 33 | 12 | - | 403 | 47 | 190 | 7 |

1. Includes all children with AIDS, or with virus detected, or with HIV-1 antibody at age 18 months or over.
2. Deaths in HIV-1 infected children without AIDS are included.
3. All were aged 14 years or under when infection was first diagnosed.

Tables 7-8 were produced through collaboration between the Institute of Child Health London, the PHLS AIDS Centre at CDSC (081 200 6868) and the Communicable Diseases (Scotland) Unit (041 946 7120). Data sources include regular returns by obstetricians to the Royal College of Obstetricians and Gynaecologists register of HIV positive pregnant women, monthly returns by paediatricians to the British Paediatric Surveillance Unit, returns by Haemophilia Centre Directors to the Oxford Haemophilia Centre (0865 225316), and voluntary confidential reports by clinicians and microbiologists to the PHLS AIDS Centre and CD(S)U.

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# PHLS-SVS
# Update on salmonella infection

October 1992 Edition 13

## Salmonella in humans

On 1 January 1992 a new reporting system for human salmonellosis came into operation within the Public Health Laboratory Service (PHLS); provisional information from this system is published here as the PHLS Salmonella Data Set. While there is a general relationship between human salmonella infection and animal carriage, the human data cannot be directly compared with the veterinary data.

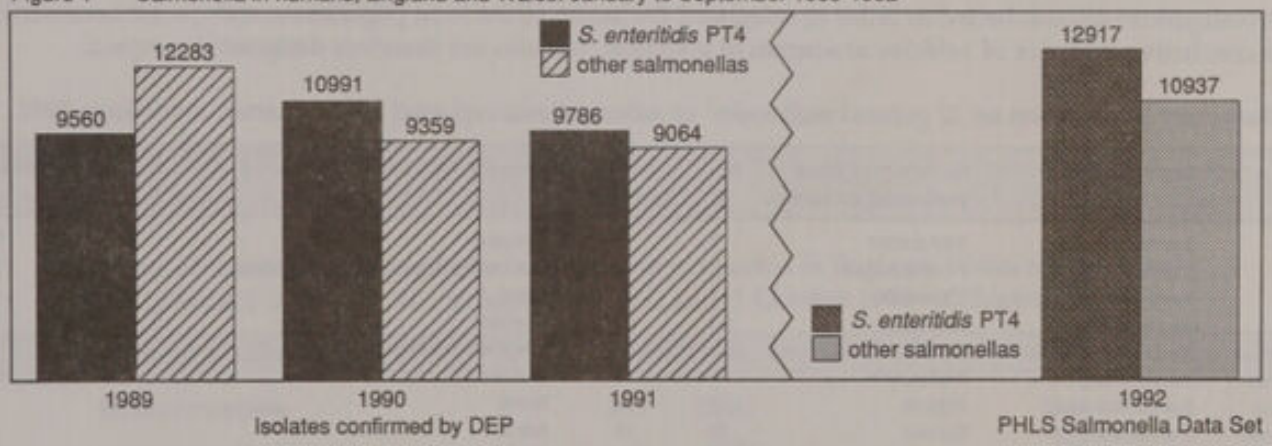
Table 1 Salmonella in humans, England and Wales, January to September 1992. PHLS Salmonella Data Set (figures in brackets are number of patients infected abroad)

| Serotype | Jan-Sept 1992 | |
|---|---|---|
| *S. enteritidis* | | |
| phage type 4 | 12917 | (1008) |
| other phage types | 2334 | (423) |
| *S. typhimurium* | 4124 | (346) |
| Other serotypes[1] | 3887 | (931) |
| *S. species*[2] | 592 | (117) |
| **All serotypes** | **23854** | **(2825)** |

Notes:
1. 'Other serotypes' consists of salmonellas fully identified by DEP as serotypes other than *S. enteritidis* or *S. typhimurium*.
2. '*S. species*' includes organisms reported without further identification, and those yet to be identified, by DEP.

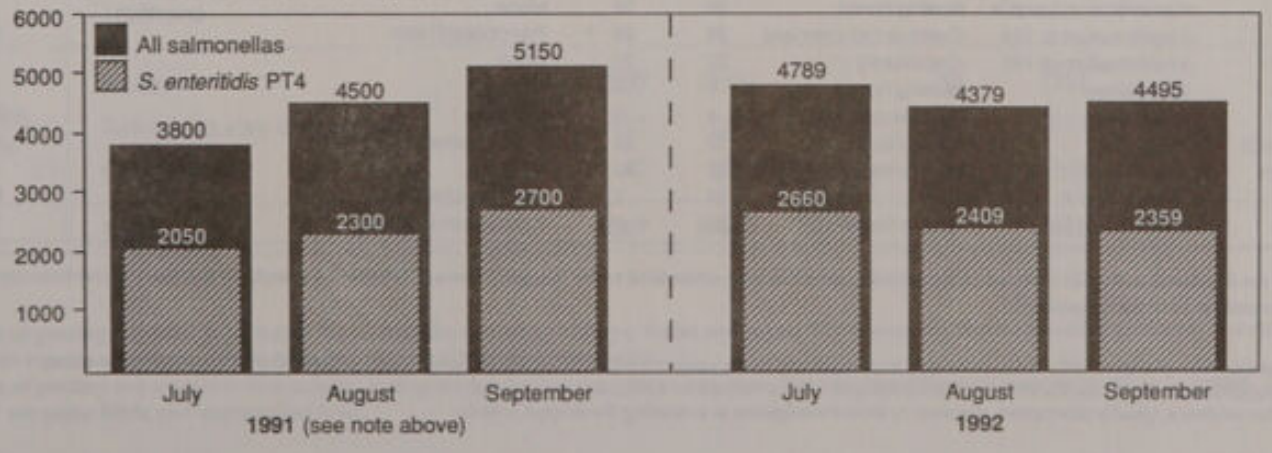
Figures from 1992 shown in Figure 1 below are not directly comparable with previous years.

Figure 1 Salmonella in humans, England and Wales: January to September 1989-1992




An attempt has been made to present the 1991 data **as if** they had been collected under the new system; this is shown in Figure 2 below. However, this cannot take account of altered reporting practices by laboratories following the introduction of the new reporting system and cannot be used as an accurate comparison of reports in 1991 with 1992.

Figure 2 Salmonella in humans, England and Wales



# Outbreaks of salmonellosis

The tables on this page summarise outbreaks of human salmonellosis reported to the PHLS Communicable Disease Surveillance Centre. On the 1 January 1992, following a recommendation of the Richmond Committee, a new system was introduced to enhance the information recorded by CDSC on general outbreaks. The new system requests additional information, including for example, the nature of the evidence on which a food is reported as the suspect vehicle of infection. This extra information is sought after receipt of the initial report and is recorded in Table 3 on outbreaks reported in the second quarter.

Table 2

Type of outbreak of human salmonellosis in England and Wales, 1 January to 30 September 1992 and (1 January to 30 September 1991)

| Outbreak type[1] | *S.enteritidis* phage type 4 | | *S.enteritidis* other phage types | | *S.typhimurium* | | Other serotypes | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| General | 69 | (57) | 11 | (16) | 14 | (18) | 19 | (12) | 113 | (103) |
| Family[2] | 645 | (262) | 110 | (60) | 192 | (92) | 190 | (55) | 1137 | (469) |
| Acquired abroad | 28 | (12) | 9 | (11) | 9 | (4) | 23 | (16) | 69 | (43) |
| Not known | - | (-) | - | (-) | - | (-) | - | (-) | - | (-) |
| **Total** | **742** | **(331)** | **130** | **(87)** | **215** | **(114)** | **232** | **(83)** | **1319** | **(615)** |

Notes:

1. An 'outbreak' represents two or more related laboratory confirmed salmonella infections in humans of whom at least one was ill or two or more related cases of illness in humans of whom at least one had confirmed salmonella infection. 'Family outbreaks' involve members of only one household. 'General outbreaks' involve the members of more than one household. Family and general outbreaks in which the infection was contracted outside England and Wales are designated 'Acquired abroad'.
2. The introduction of the new common laboratory report form has resulted in enhanced ascertainment of family outbreaks.

Table 3

Local investigations are conducted in order to safeguard the health of the local population, and do not necessarily provide conclusive evidence of vehicles or sources of infection. Vehicles are therefore designated 'suspect'.

Summary information on 22 general outbreaks† of salmonellosis reported in the quarter, April-June 1992

| Region | Organism | Location of food prepared or served | No. ill | Cases positive | Suspect vehicle | Evidence |
|---|---|---|---|---|---|---|
| Yorkshire | *S.enteritidis* pt 4 | Restaurant | 9 | 6 | Chicken | M |
| Trent | *S.enteritidis* pt 4 | Private house | 6 | 5 | Lemon meringue pie, homemade | D |
| Trent | *S.enteritidis* pt 4 | Takeaway | 11 | 11 | None | - |
| E.Anglia | *S.enteritidis* pt 4 | University | 58 | 43 | Chicken | S |
| N.W.Thames | *S.enteritidis* pt 4 | Commercial premises | 40 | 30 | Coronation chicken and rice | S |
| N.E.Thames | *S.enteritidis* pt 4 | Restaurant | 9 | 5 | Tiramisu | D |
| S.E.Thames | *S.enteritidis* pt 4 | School | 27 | 14 | None | - |
| S.W.Thames | *S.enteritidis* pt 4 | School | 38 | 18 | None | - |
| Wessex | *S.enteritidis* pt 4 | Guest house | 34 | 23 | Cold meats | D |
| Wessex | *S.enteritidis* pt 4 | Restaurant | 4 | 3 | Egg fried rice | D |
| S.Western | *S.enteritidis* pt 4 | Private house | 13 | 10 | Prawn fried rice, crab meat fried rice, beansprouts in curd | M |
| Wales | *S.enteritidis* pt 4 | Private house | 26 | 10 | Scotch egg | S |
| Northern | *S.enteritidis* pt 7A | Nursing homes | 29 | 15 | Banana custard meringue | S |
| Mersey | *S.enteritidis* untypable | Nursing home | 39 | 39 | None | - |
| Wales | *S.typhimurium* dt 124 | Commercial premises | 28 | 28 | Pre-cooked ham | D |
| E.Anglia | *S.typhimurium* dt 193 | Community | 22 | 22 | None | - |
| Yorkshire | *S.bredeney* | Nursing home | 9 | 1 | None | - |
| Northern | *S.bredeney* | Takeaway | 6 | 4 | Chicken | D |
| W.Midlands | *S.panama* | Market stall | 32 | 32 | Beef, chicken and pork | M |
| S.Western | *S.saint-paul* | Private house | 72 | 60 | Turkey | S |
| Wales | *S.virchow* pt 4 | Club | 38 | 2 | Chicken pieces | M |
| E.Anglia | *S.virchow* pt 8 | Private house | 30 | 6 | Coronation chicken | S |

† One of the 34 general outbreaks reported in the second quarter has been reclassified to the "Acquired abroad" category. A completed summary report form has not been provided for 11 of the remaining 33.

Notes:

M = identification of a salmonella of the same type from cases and in the suspect vehicle, or vehicle ingredient/s; or other strong microbiological evidence
S = a significant statistical association between consumption of the suspect vehicle(s) and being a case.
D = other evidence, usually descriptive, reported by local investigators as indicating the suspect vehicle.

# Salmonella in animals

The Zoonoses Order 1989 requires that all isolations of salmonella from animals must be reported to a veterinary officer of the Ministry of Agriculture, Fisheries and Food. Salmonella may be isolated from animals (including birds) which show signs of disease or which are clinically healthy, whether investigated for statutory purposes or otherwise.

Table 4 Incidents[a] of salmonella in farm animals reported in England, Wales and Scotland in the period 1 October 1991 - 30 September 1992 and (1 October 1990 - 30 September 1991)

| Serotype | Cattle | | Sheep | | Pigs | | Poultry[b] | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| *S.enteritidis* | | | | | | | | | | |
| all phage types | 21 | (23) | 3 | (6) | 2 | (0) | 867 | (953) | 893 | (982) |
| phage type 4[c] | 8 | (5) | 4 | (1) | 1 | (0) | 392 | (183) | 405 | (189) |
| *S.typhimurium* | 359 | (552) | 29 | (40) | 101 | (88) | 349 | (241) | 838 | (921) |
| Other serotypes | 648 | (692) | 80 | (124) | 113 | (149) | 1410 | (1379) | 2251 | (2344) |
| **All serotypes** | **1028** | **(1267)** | **112** | **(170)** | **216** | **(237)** | **2626** | **(2573)** | **3982** | **(4247)** |

**Notes:**

a. An "incident" refers to an isolation or isolations of salmonella from either an individual animal or from one or more animals in a group, on one or more occasions.

b. "Poultry" comprises domestic fowls, turkeys, geese, ducks, guinea fowls, pheasants, partridges, quail and pigeons.

c. Figures show only those isolates so far phage typed. Figures for 'last' period show only those for which results were available at the time.

# Salmonella in poultry

Table 5 Incidents of *S.enteritidis* or *S.typhimurium* in poultry reported in England, Wales and Scotland in the period 1 October 1991 - 30 September 1992 and (1 October 1990 - 30 September 1991)

| | Type of poultry | *S.enteritidis* | | *S.typhimurium* | |
|---|---|---|---|---|---|
| See (a) below | Broiler breeders | 243 | (256) | 37 | (24) |
| | Layer breeders | 9 | (15) | 1 | (2) |
| | Commercial layers (caged) | 60 | (44) | 15 | (18) |
| | Commercial layers (others) | 23 | (34) | 9 | (7) |
| See (b) below | Broiler | 359 | (532) | 52 | (25) |
| | Turkeys, ducks and geese | 131 | (21) | 161 | (51) |
| | Other birds | 42 | (51) | 74 | (114) |
| | **Total** | **867** | **(953)** | **349** | **(241)** |

**Notes:**

a. Types of poultry required to be tested for salmonella excluding turkeys, ducks and geese. The figures for broiler breeders and layer breeders include reports of isolations from breeding flock and hatchery monitoring.

b. Types of poultry not subject to compulsory testing except for turkeys, ducks and geese kept for breeding which are required to be tested. 'Other birds' are game birds and unclassified fowl.

# Compulsory slaughter of flocks

Poultry flocks compulsorily slaughtered in England, Wales and Scotland under the Zoonoses Order 1989 since 1 March 1989

Table 6

| Type of poultry | *S.enteritidis* | *S.typhimurium* | Total |
|---|---|---|---|
| Broiler breeders | 71 | 2 | 73 |
| Layer breeders | 13 | 4 | 17 |
| Commercial layers (caged and others) | 213 | 46 | 259 |
| **Total** | **297** | **52** | **349** |

Figure 3

*S.enteritidis* infected commercial laying flocks compulsorily slaughtered
**Quarterly** summaries 1989-1992

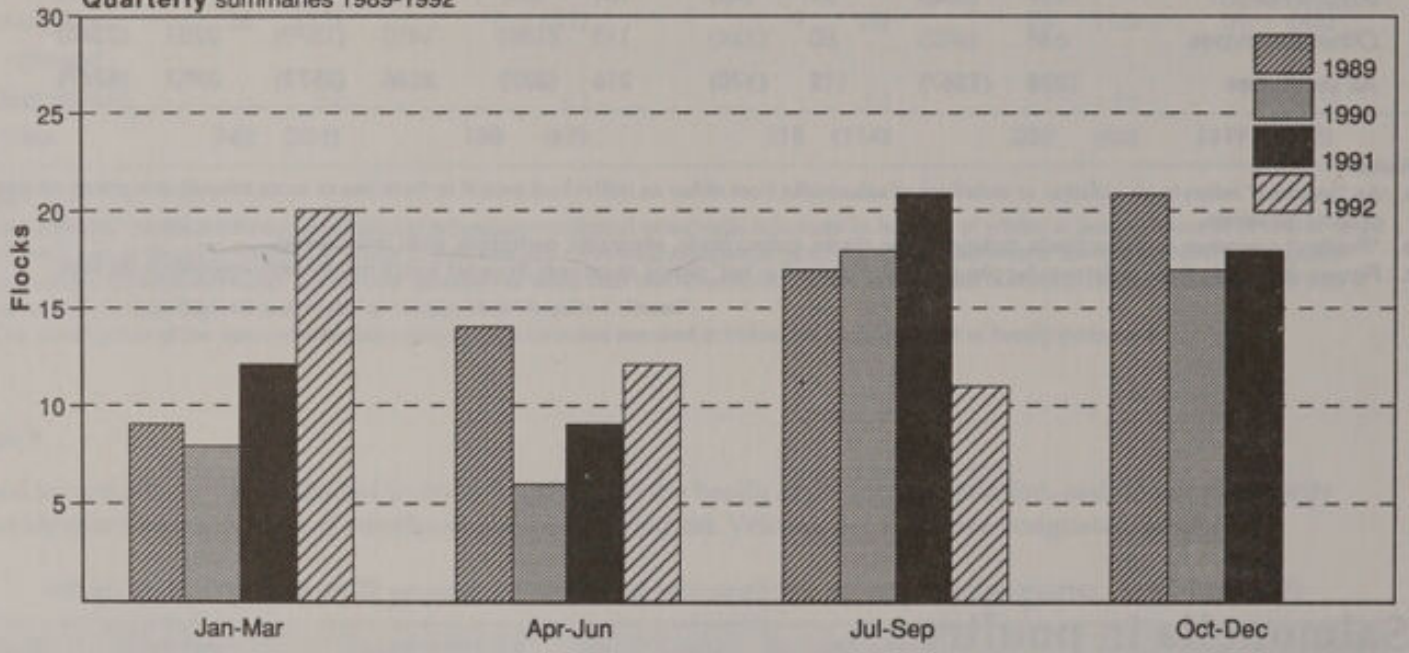
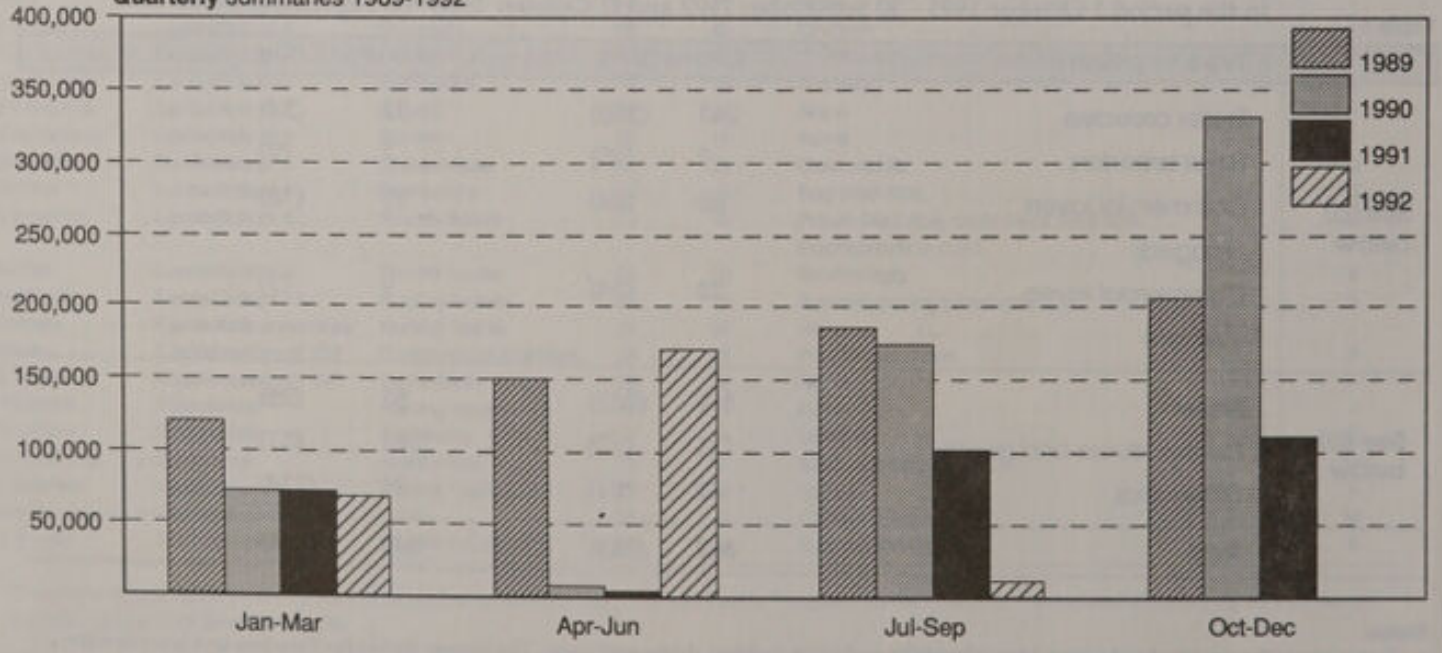



Figure 4

*S.enteritidis* commercial laying birds from infected flocks compulsorily slaughtered
**Quarterly** summaries 1989-1992

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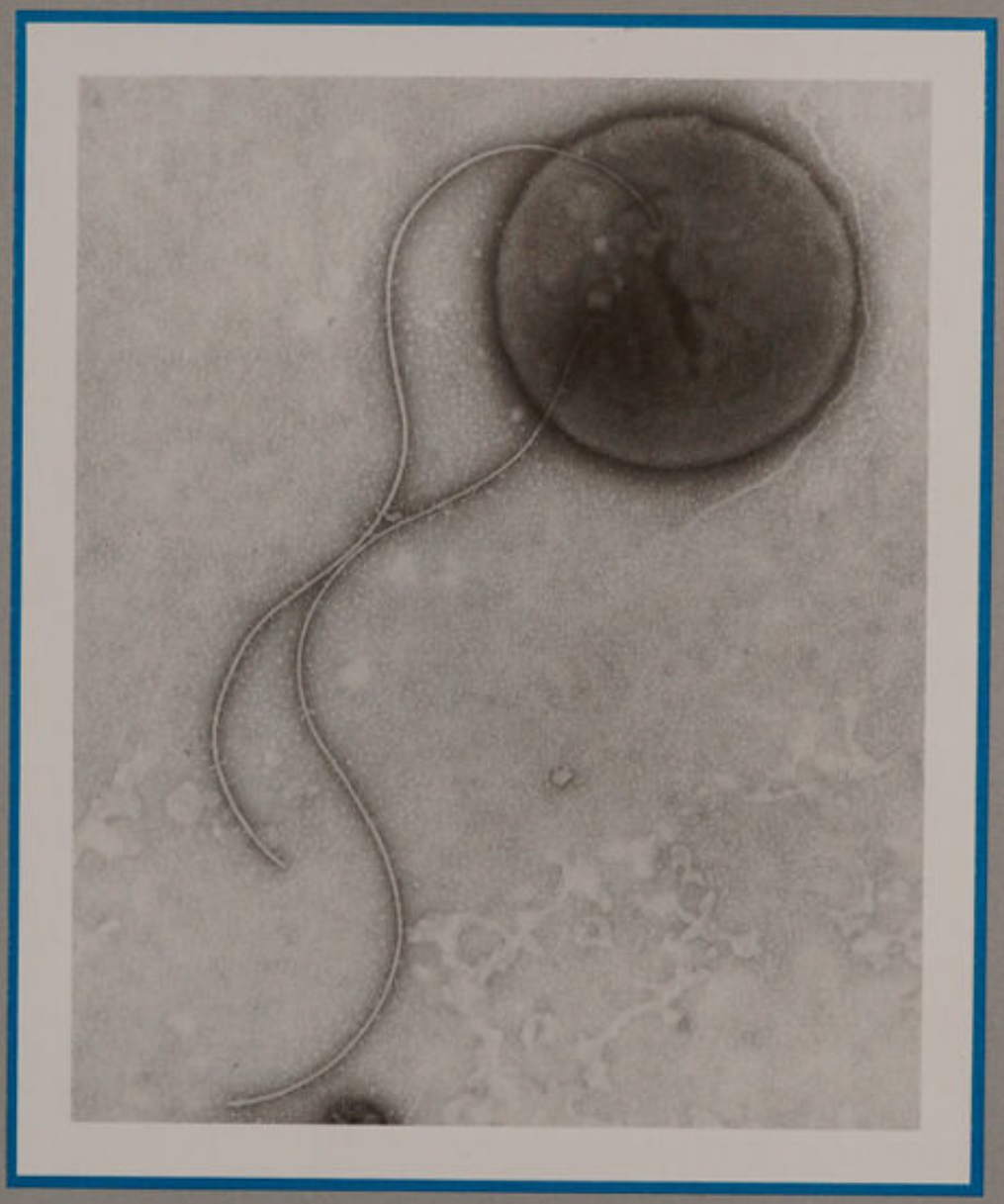

PHLS

Quarterly from
The Public Health Laboratory Service

# EDITORIAL PANEL



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Articles, news items, letters to the editor and books for review are welcomed. Notes for contributors appear on page 96.

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**Cover illustration**

Development of the coccal form of *Campylobacter jejuni* occurs when conditions become relatively unfavourable for growth. The cells lose the terminal inner membrane thickenings associated with the flagellar insertion, and develop the spherical shape by expansion of one side of the spiral cell. In this way the two poles, and therefore the flagellar insertions, come to lie on the same side of the coccus. This appears to be an active process, requiring energy, and older, longer vibroid cells in a culture do not undergo this transformation.

Coccal forms are rapidly produced in cultures left on the laboratory bench, perhaps by the influence of fall in temperature as well as exposure to air. In an aqueous environment young cells become coccal and many of these persist for long periods. Though they can no longer be cultured they are still able to colonise mice.[1]

(Electron micrograph of a coccus (1.0 µm in diameter) from a three day culture of *C.jejuni*, negatively stained using phosphotungstic acid. Magnification x 46 000. Provided by Alan Curry, Manchester Public Health Laboratory.)

1 Jones DM, Sutcliffe EM, Curry A. Recovery of viable but non-culturable *Campylobacter jejuni*. *J Gen Microbiol* 1991; **137**: 2477-82.

# CONTENTS

## Articles



## Conference Reports

# Computer-assisted monitoring of frequencies of organisms reported to CDSC

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**CP Farrington**
PHLS Statistics Unit & CDSC Immunisation Division

## Introduction

This article describes a project for monitoring changes in the frequency of organisms reported to CDSC. Information relating to expected and observed frequencies can be obtained for all organisms reported during a specified week, typically the most recent week. Users can choose to display or print out graphs of occurrences of organisms over a specified number of years prior to the specified week. This is done by selecting graphs from a table of organisms ranked alphabetically or by exceedance score, the exceedance score being a measure of the unexpectedness of the organism's frequency in the current week by comparison with its occurrence in previous weeks at the same time of the year.

Ultimately, the application is intended as a mechanism for helping staff in the detection of outbreaks. It runs in an OpenWindows environment with the Oracle relational database application for CDSC.

## Summary tables of frequencies

Two master matrices or tables of organism counts have been set up for retrieval of the relevant organism frequencies. These tables contain the frequency of every organism reported to CDSC since the beginning of 1985. One table contains the frequencies distributed across 21 geographical regions and the other contains the frequencies distributed across five age groups. The system currently provides information relating to total frequencies. Future enhancements will provide analyses of the different regional and age group counts.

Data since 1985 may be subdivided into three classes as follows:

1) data prior to 1989 (the 'North West Thames data')
2) the 1989 data
3) data for 1990 onwards.

Data prior to 1989 was captured before installation of the Oracle RDBMS (Relational Database Management System) and had to be translated into RDBMS tables before the frequencies of organisms per CDSC week could be inserted into the summary tables required for the project. Data for 1989 and 1990 onwards has been retained in two separate databases and summary frequencies have been extracted by programs reading from the two different databases.

## Changes to the organism descriptions and codes

A completely new set of organism codes with, in some cases, revisions to organism names was introduced at the beginning of 1990. Consequently, a linking mechanism had to be prepared so that meaningful comparisons could be made of organism counts before and after the beginning of 1990. A program was written to establish links automatically between the two sets but considerable manual editing of the link table is still required, for example where spelling changes to organism names have been made. An easy-to-use linking form has been designed for this purpose.

Revision of the organism coding system has in some cases resulted in old categories being split into two or more new categories. We decided to utilise frequency information prior to 1990 for comparisons with any current organism occurrence only when a one-to-one relationship could be established between the organism's present code and any corresponding pre-1990 code. Nevertheless, data for every organism code since 1985 has been captured in the regions and age range summary tables. A mechanism triggered by the computer's clock automatically adds a new set of organism frequencies to the summary tables every week.

## Identifying organisms with unusually high frequencies

Having produced summary tables of organism frequencies and a mechanism for relating the pre-1990 and post-1989 organism counts, a program retrieves values for specified organisms during specified weeks from the beginning of 1985 until the current week. Frequency of reporting of many organisms exhibits seasonal variation, typically over an annual cycle. For this reason, the count expected for the current week is calculated using organism counts in baseline weeks neighbouring the current week in previous years. The user may select both the number of years back and the window of neighbouring weeks.

Upper and lower prediction limits above and below the expected frequency are computed to allow for random variation of the weekly count around the expected value. The

exceedance score is a measure of the amount by which the observed count during the most recent weeks exceeds the upper prediction limit.

### Menu options

The 'front-end' menu of the application provides simple options for interactive selection of the facilities available. The main menu presents the user with options for starting a report, printing a finished report, and displaying or printing graphs of the organisms reported by week.

Examples of the output of the system are shown opposite. Figures show data for five years up to and including the week beginning 7 June 1992.

### How the system works

The main reporting mechanism queued from the Start Report option takes as arguments the current week, the number of years back for which the user wishes to compute values, the extension or window for establishing 'baseline' values and the confidence threshold. The program creates a vector of consecutive weeks for the period specified, taking into account whether any particular year contains 52 or 53 weeks. A parallel vector of indicator bits is set up. This stipulates which weeks are to be used as baselines in the calculation of exceedance scores. The program allows ranges of baseline weeks to straddle year boundaries.

An array of organisms is produced containing the present organism names and codes together with their frequencies for the current week, these records being selected in descending order of frequency. For each organism selected from the current week, a triple of vectors corresponding to the vector of consecutive historical weeks is produced. The triple contains columns for the organism frequency, week number and indicator bit.

A search is carried out through the table that links the organisms in the pre-1990 and post-1989 sets. If a corresponding pre-1990 organism code can be found, it is used for read-

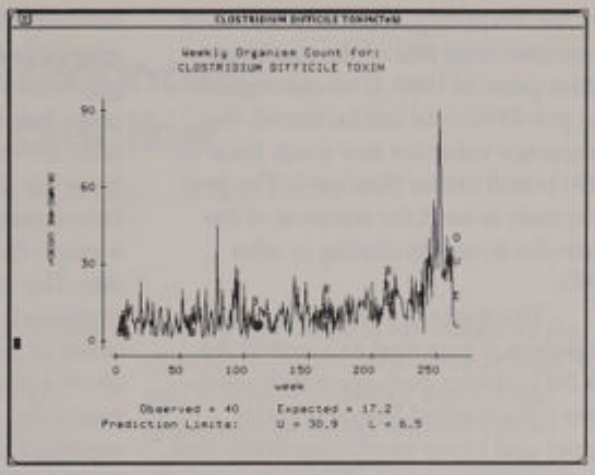



*Figure 1* A graph of *Clostridium difficile* displaying reported weekly frequency over time.

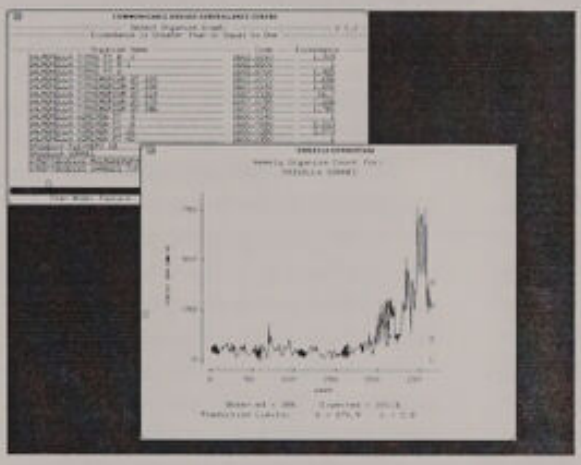


*Figure 2* The user may display any graph of an organism reported in the selected week from menus of organisms sorted either by exceedance score or alphabetically.

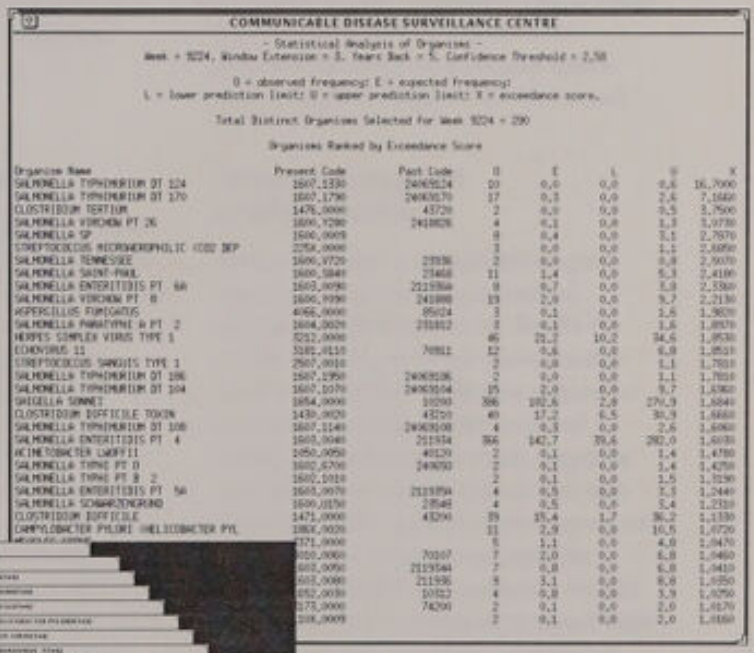



*Figure 3* The beginning of a report showing organisms for a selected week ranked by exceedance score.

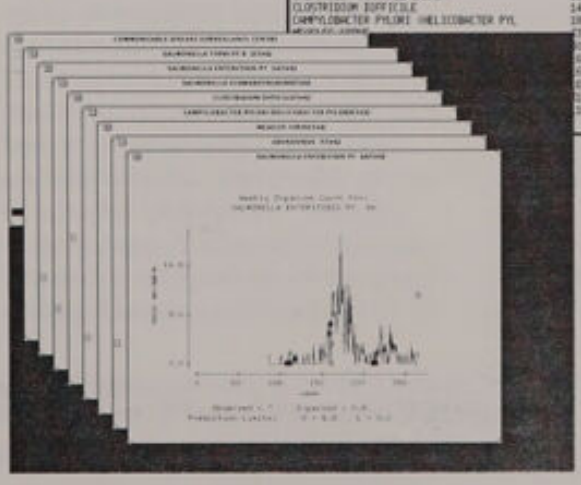


*Figure 4* The Windows facility allows several graphs to be displayed on the screen at once. Graphs may be selected or deselected by using a mouse.

ing the frequency of occurrence of the organism from one of the summary tables prior to 1990. If no corresponding pre-1990 code can be found, the frequency value for any week prior to 1990 is null rather than zero. The present code is used for retrieval of frequencies in weeks during or after 1990.

The triple of vectors for each organism is supplied as input to the GLIM statistics package which calculates the expected frequency, the upper and lower prediction limits and the exceedance score. GLIM also generates for each organism another triple of vectors which is used as input to the graphics component of MINITAB. The control program then executes MINITAB which generates a graph file for each organism. Once all organisms selected from the current week have been processed in this way, the main program disconnects from the database, having produced a two-dimensional array or matrix and a graph file for each reported organism. The rows of the matrix are the organisms in the current week in order of frequency, and the columns are the organism name, present code, past code, observed frequency, expected frequency, lower prediction limit, upper prediction limit and exceedance score for the arguments supplied.

The matrix is then sorted twice, by exceedance score and by alphabetical order of present organism name. Each sorted matrix is then written to a file which may be printed when the job is finished.

## Summary

The application described is a fully functional mechanism for computer-assisted monitoring of organism occurrences. Currently the interactive menu may be used to produce printed reports and graphs showing organism counts over time-spans and with thresholds specified by the user. Future enhancements will include graphs and print-outs of organism counts for selected regions and age ranges.

# Towards error free HIV diagnosis: notes on laboratory practice

*(These notes were drafted following a meeting on 11 March 1992 of the PHLS AIDS Diagnosis Working Group* and have been endorsed by it.)*

## Procedure

Most mistakes in HIV laboratory diagnosis arise from procedural errors such as mislabelling, misplacing specimens in a rack or microplate, cross-contamination due to carry over on a tip or by splash, faulty transcription of results or bad communication. There are at least seven stages at which these errors can arise, namely:

- Specimen collection
- Specimen log-in
- Separation of serum
- Transfer into test rack/plate
- Reading test results
- Transcription to the report
- Communication of the report to clinician

These, and not the assay methods themselves, are the most error-prone aspects of HIV testing and they therefore deserve most attention when attempting to improve laboratory practice.

## The initial anti HIV test

The range of 'screening' assays available is now very wide and includes several excellent combined HIV 1/2 EIA and some simple or rapid tests such as Serodia-HIV, HIVCHEK 1+2 and Testpack. The choice between them depends on the skills and equipment available, the number of specimens to be tested and cost. In competent hands the sensitivity of most of these assays is adequate and similar. Each may therefore be used singly to generate a negative report. The clinician should, however, be made aware if the initial test is only sensitive to anti HIV 1 as there may be reasons (eg previous residence in West Africa) why an assay sensitive to anti HIV 2 is needed. Otherwise, unless there is clinical suspicion of recent infection, the only advantage of doing two assays is as a check of procedure. For this the second test should whenever possible be done on serum taken from the clot in the original specimen container. When a second test is not done testing procedure must be particularly scrupulous to avoid false negative reports due to technical or clerical error.

## Identifying an initial reaction as a false positive

Initial assay (A, Figure) positive anti HIV reactions may be false or true. In attempting to determine this little is to be gained by repeating the test by the same assay though it may be convenient and reassuring to do so at some stage. A better course is to test the specimen by further screening assays (B, C, Figure) either in the same laboratory or at another laboratory where confirmatory testing is routinely done. There are two common outcomes of this: either the two further assays are both unreactive or (see below) they are both reactive. The outcome A+, B-, C- is highly suggestive of false positivity in assay A, especially if the reaction in A is unrepeatable. If the optical density/cut-off ratio for assay A is <2 and the individual is not stated on the clinician's request form to be at high risk, it is recommended that a negative report be issued without follow-up. (This is a pragmatic course based on UK experience and takes account of the anxiety that delay in reporting in these circumstances inevitably gives rise to.) Otherwise testing of a follow-up specimen collected after a minimum interval of 14 days to rule out recent seroconversion is recommended. A shorter interval might not take account of the slight differences in sensitivity at seroconversion of available screening assays. When the follow-up specimen has been obtained it should be tested *in parallel* with the original specimen by assays A, B and C. It is suggested that if identical or less reactive results are obtained in tests on a second specimen a negative report can be issued.

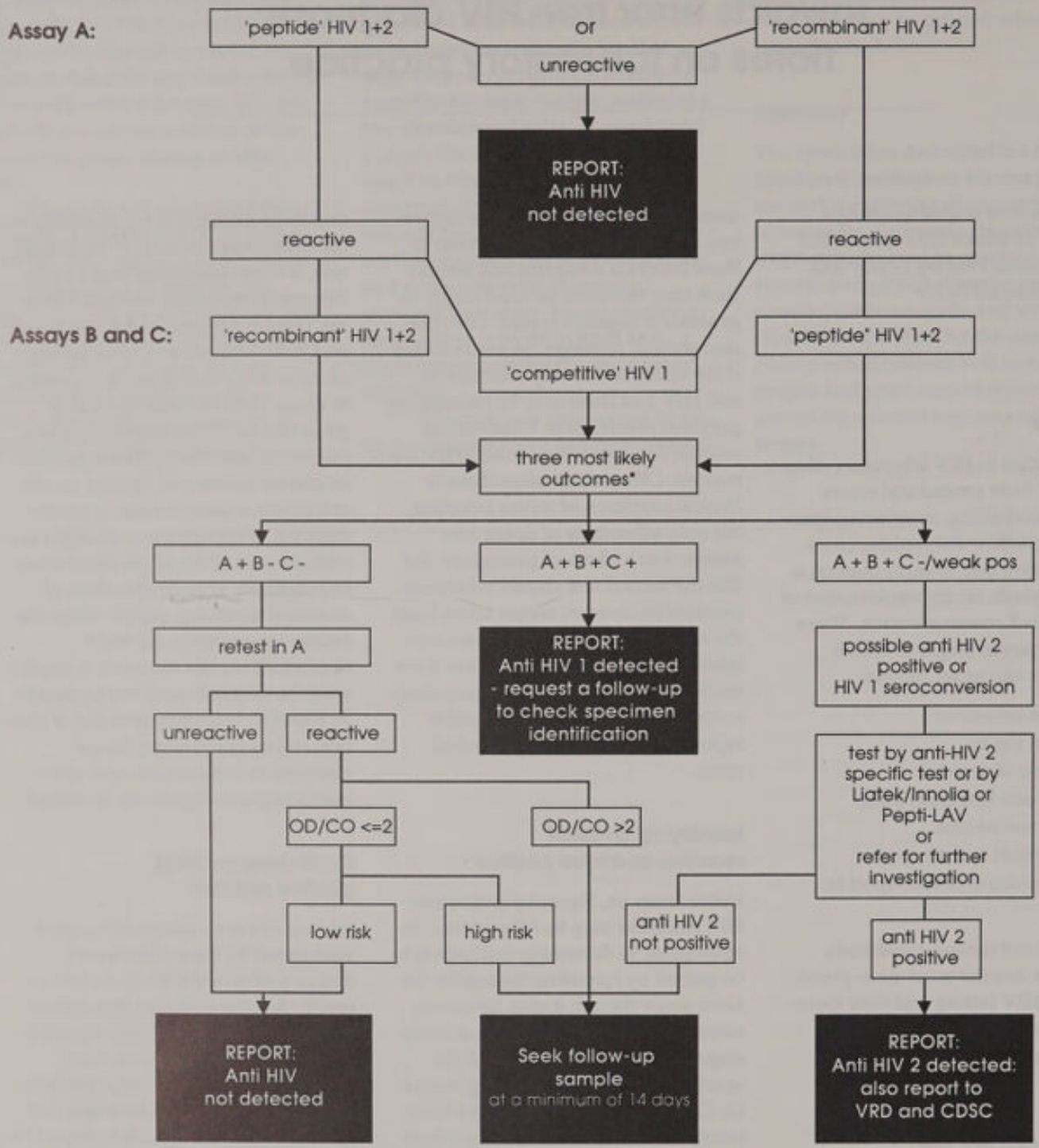
## Confirming an initial positive reaction

Almost always a seropositive specimen tested by three commercial assays will give rise to an A+ B+ C+ result. As long as these three assays have been chosen with due regard to diversity in antigen source (viral lysate, recombinant protein, peptide, see Table) and assay format this outcome is a basis for a positive report *on that specimen*. Whenever possible, though, a follow-up specimen should immediately be obtained to check that no procedural error has occurred. A single test on this is all that is required (as long as it is reactive) but until a second specimen has been checked misidentification of the first specimen remains a possibility.

*The current membership of the PHLS Aids Diagnosis Working Group is: A Codd, J Connolly, J Craske, U Desselberger, R Eglin, E Follett, J Hawkins, J Kurtz, P Mortimer (Chairman), J Parry, A Roome, D Samuel, S Skidmore, R Tedder.

Correspondence should be addressed to Dr P Mortimer, Virus Reference Division, PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK.

Figure **An algorithm for anti HIV confirmatory testing**




* If other combinations of results arise it is recommended that the sample(s) be referred to one of the confirmatory laboratories.

**Suggested wording for reports**

i) Initial test negative= HIV antibody NOT detected

ii) (for laboratories offering one assay only)
Initial test positive = Preliminary report: anti HIV screening test reactive confirmatory results to follow: please obtain a second specimen.

iii) A + B - C - with repeat A - = HIV antibody NOT detected

iv) If the A + is referred from another laboratory and the results are:
A + B - C - repeat A + OD/CO <2 = Give the individual assay results and if a low risk situation report: HIV antibody NOT detected. If a high risk situation seek a follow-up specimen at 14 days or more.

v) A + B - C - repeat A + OD/CO >2 = Give the individual assay results and seek a follow-up specimen at 14 days or more.

vi) A + B + C + = Preliminary report: HIV 1 antibody detected, please obtain a repeat specimen to check identification.

vii) Specimen 1 A+B+C+ Specimen 2 A + = HIV 1 antibody detected in both specimens.

### Recognition of HIV 2 infection

If one of the three reactive screening assays is a competitive assay incorporating HIV 1 antigen and its reactivity is not unexpectedly low, HIV 1 infection can be assumed. In the case of a weak or negative reaction HIV 2 infection or a seroconversion may be suspected. A 'line assay' such as Liatek/Innolia may be usedto discriminate between HIV 1 and HIV 2 infection. The cost of using Western blots to make this distinction is unjustifiably high and the results often not conclusive, so either Liatek/Innolia should be used or the specimen sent to a reference laboratory for tests that will discriminate between HIV 1 and HIV 2. Reference laboratories should also be used when other discrepant or anomalous findings arise. Suspected anti HIV 2 positive sera referred to the PHLS Virus Reference Division (VRD) can be tested there by a native antigen-based Western blot. It is particularly important to notify VRD and the PHLS Communicable Disease Surveillance Centre of cases of HIV 2 infection as some other methods of HIV surveillance in the UK (eg screening of infants dried blood spots) are insensitive to anti HIV 2 and it may as a result be falsely assumed that HIV 2 infection continues to be very rare.

### Testing after exposure to HIV

A common request is to test an individual who may have recently been exposed to HIV. Unfortunately there is no rapid way of excluding the possibility that transmission has occurred, and no practical alternative to an anti HIV test done at an interval

Table **HIV antigens employed in commercial immunoassays**

| Antigen type | Manufacturer (supplier) | Order code | Test | Antigen |
|---|---|---|---|---|
| **Native** | Behring | OUVA 12/13 | Enzygnost Micro HIV 1 (competitive EIA) | HIV 1 viral lysate |
| | Diagnostics Pasteur (NBL) | 72 237 | Rapid'Elavia Mixt | HIV 1/2 purified, inactivated |
| | Organon Teknika | 6005 | Vironostika HIV Mixt | Purified HIV 1 viral lysate, HIV 2 peptides |
| | Fujirebio (Mast) | 9230* 9235 | Serodia-HIV (gel particle agglutination) | HIV 1 inactivated, from cell culture extract |
| **Recombinant** | Abbott | 1458-24* 1458-32 | Recombinant HIV 1 EIA | Recombinant (HIV 1 core and env) |
| | Abbott | 1A80-24* 1A80-32 | Recombinant HIV 1/HIV 2 EIA | Recombinant (HIV 1 core and env, HIV 2 env) |
| | Abbott | 3A10 | Recombinant HIV 1/HIV 2 EIA, 3rd generation | Recombinant (HIV 1 env and gag, and HIV 2 env) |
| | Abbott | 1A83 21 | Testpack HIV 1/HIV 2 | Same as recombinant HIV 1/2 kit |
| | Sorin (Incstar) | P3076 | ETI-Ab-HIV 1/2 | Recombinant (HIV 1 p24, gp41), synthetic peptide (HIV 2 gp36) |
| | Wellcome | VK56* VK57 | Wellcozyme HIV Recombinant (competitive EIA) | Recombinant (HIV 1 core and env) |
| | Wellcome | VK54* VK55 | Wellcozyme HIV 1+2 Recombinant | Recombinant (HIV 1 core and env) and synthetic peptide (HIV 2 env) |
| | Innogenetics (Mercia) | M422 | Innotest HIV 1/HIV 2 Ab | Recombinant (HIV 1 env and core) and synthetic peptide (HIV 2 env) |
| | Ortho | | HIVCHEK 1+2 | Recombinant (HIV 1 env) and synthetic peptide (HIV 2 env) |
| **Synthetic peptide** | Behring | OWRP 10/11 | Enzygnost HIV 1/2 EIA | HIV 1 and HIV 2 synthetic peptides |
| | IAF Biochem (Bio-stat or Launch) | B-001/2 | Detect-HIV | HIV 1 and HIV 2 synthetic peptides |
| | Diagnostics Pasteur (Ortho) | 72 266* 72 267 | Genelavia Mixt | Synthetic peptides (HIV 1 env and HIV 2 env), recombinant gp 160 (HIV 1 and HIV 2) |

* Where product code numbers are given, this denotes that two sizes of kit are available. The former indicates the 100 test kit, the latter a larger size.

Note: it is most inadvisable to base confirmatory testing on combinations of screening assays that incorporate antigen of the same origin, eg all recombinant.

of at least three months. Though available data are meagre it is believed that more than 90% of HIV seroconversions take place within three months of exposure, no matter how this occurs. If, therefore, an anti HIV screening test is negative at that interval the patient may be reassured. The three-month interval represents a compromise between certainty that transmission has not occurred and a wish to be able to reassure the patient as soon as possible. In several years of operating the 'three month rule' in the UK no instances where it has proved misleading have come to the attention of the PHLS AIDS Laboratory Diagnosis Working Group.

### Rapidity of testing

Laboratories are under increasing pressure to report test results rapidly, and in some localities a same day service is sought for some individuals. As has already been noted, a *carefully* performed single assay which gives a negative reaction is generally a basis for a confident negative report: same day reporting is therefore feasible in most cases. It must, however, be explained during counselling prior to an initial test that a second specimen and further testing may be needed and that this does not necessarily carry an implication of seropositivity. This is especially important for same day testing which may also give rise to difficulty in communicating results speedily and correctly. In addition, attention must be given to preserving confidentiality, eg when telefaxing or telephoning results.

As regards the speed of confirmatory testing, laboratories undertaking confirmatory testing should aim to complete and report a result based on three assays within five working days. However, circumstances that give rise to a need for extra tests may entail some delay. It is expensive and inimical to accuracy to do special HIV tests on a 'one off' basis, and when confirmatory laboratories do have to batch these tests there may be a slight delay in reporting a result. In such circumstances consideration should be given to issuing a provisional report, perhaps by telephone.

### Status of other HIV assays

Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific, and they are expensive. In selected circumstances (eg HIV diagnosis in infancy) amplification of HIV DNA by PCR and tests for specific IgA anti HIV and HIV p24 antigen may be helpful. Specimens for these tests should be forwarded to a reference laboratory, following a preliminary telephone call to discuss the case, establish what are the correct specimens to collect and ensure that the appropriate tests are currently available.

### Uses and accuracy of tests on non-serum specimens

Screening for non-clinical purposes, eg anonymised testing as part of epidemiological surveys, does not require standards of accuracy quite as high as clinical diagnostic testing. Nevertheless, especially in a low prevalence population such as in the UK, high specificity and reasonable sensitivity are important. Tests on dried blood spot eluates, saliva and urine specimens that are accurate enough for epidemiological purposes have been introduced, but they are not yet in use for clinical testing. With a check of the IgG content of the specimens, they would be satisfactory for that purpose in circumstances where blood might be difficult to obtain.

### Wording of reports

It is particularly important that reports on HIV tests should be considered, carefully formulated and clearly expressed. The suggested wordings for reports set out beneath the Figure may be useful.

### Conclusion

It is now clear that PHLs and other NHS laboratories, working in collaboration with reference centres, can offer accurate, rapid and economical HIV testing mostly based on commercial screening assays for anti HIV. The quality of these assays is now so high that any further improvements in laboratory performance are likely to come from better procedures rather than a better selection of laboratory assays.

The PHLS AIDS Laboratory Working Group will keep HIV testing practice under annual or if necessary more frequent review and update these notes. Through its chairman, it welcomes comments and criticisms, as well as any data that supports or challenges the assumptions implicit in this guidance.

### A final warning

Within the last year incidents have been recognised in which carry over between specimens or between adjacent wells on microtitre plates has occurred. Current anti HIV assays are so sensitive that even trace carry over of material can lead to a strong false positive result. This is a further reason to check every positive result on a follow-up specimen.

### Selected references

Three recent papers on the subject of cost-effective laboratory confirmation of HIV infection by the use of combinations of anti HIV screening assays may be of interest, as may a paper describing a 'carry over' incident.

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# Pyrolysis mass spectrometry of micro-organisms

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Pyrolysis of micro-organisms yields a complex mixture of products, the pyrolysate, which reflects the overall organic composition of the cells and the pyrolysis conditions. Such pyrolysates can be analysed quantitatively by mass spectrometry and the resulting mass spectra (Figure 1) represent transient 'bacterial fingerprints' which can be compared mathematically for relatedness. Thus pyrolysis mass spectrometry (PyMS) has considerable potential for identification, classification and typing of bacteria with a high degree of discrimination.

Early studies on PyMS of micro-organisms were somewhat disappointing. They were hampered by expensive, cumbersome hardware and lack of suitable computer software to fully analyse the complex pyrolysis data. Manual loading of samples coupled with a prolonged processing time meant that PyMS was labour-intensive with a low sample throughput per day. Attempts to compare new PyMS data with that accumulated in data libraries were frustrated by inherent machine instability leading to poor reproducibility over time. In collaborative inter-laboratory studies of bacterial identification PyMS failed to yield consistent results and the technique fell into disrepute. The recent development of low-cost, fully automated pyrolysis mass spectrometry[1] coupled with sophisticated statistical software has led to a re-appraisal of PyMS for the characterisation of micro-organisms.[2-4]

The apparatus, technique and principles involved in PyMS have been documented elsewhere.[1,2] On each occasion all isolates to be compared by PyMS are cultured on a suitable non-inhibitory medium for a standard length of time and pyrolysed as a single batch. In this laboratory, duplicate subcultures of each isolate are prepared, usually on solid media, and pyrolysed separately to give a measure of machine reproducibility. Samples of representative colonies from each subculture are smeared onto V-shaped Ni-Fe pyrolysis foils held in pyrolysis tubes. Triplicate samples of each subculture are usually prepared. The foils are dried at 80°C for 5 minutes and pyrolysed at 530°C on a PYMS 200X pyrolysis mass spectrometer (Horizon Instruments). The analysis of each sample takes approximately 1 1/2 minutes and the machine can analyse 300 samples per batch. Integrated ion counts at unit mass intervals of 51 to 200 are recorded on floppy disk together with the pyrolysis sequence number and total ion count for each sample.[5,6]

The triplicate samples from each subculture are labelled as a single group and discriminative analysis is performed on ion counts normalised to account for variation in sample size. The mass ions with the greatest ratio of between-group to within-group variations are subjected

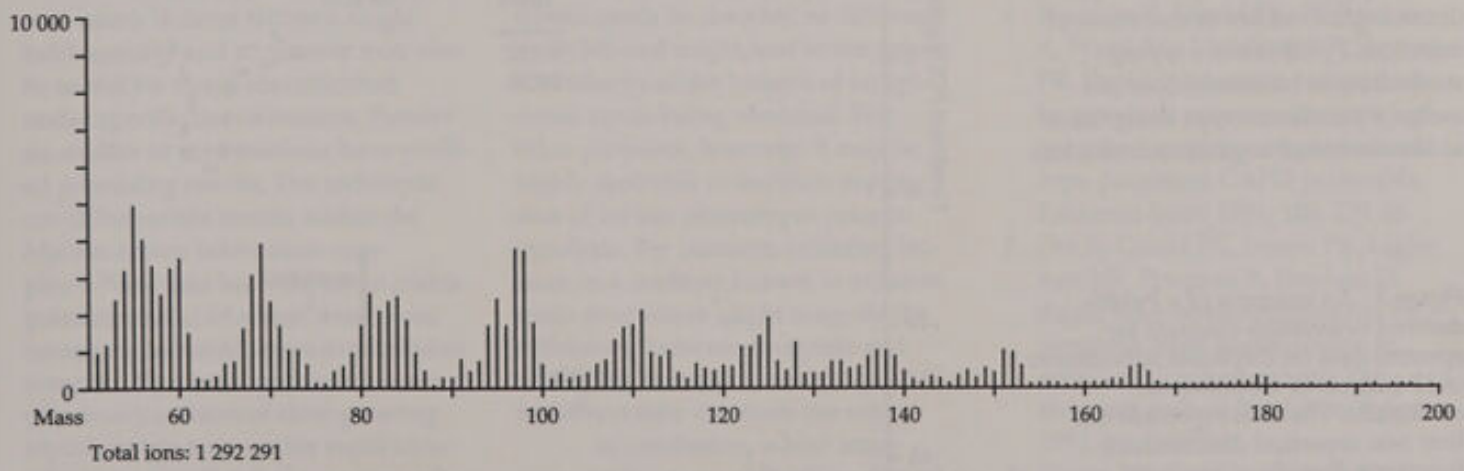


*Figure 1* An example of a mass ion spectrum obtained by pyrolysis mass spectrometry of bacteria. Integrated ion counts at unit mass intervals from 51 to 200, and the total ions obtained from the sample, are displayed.

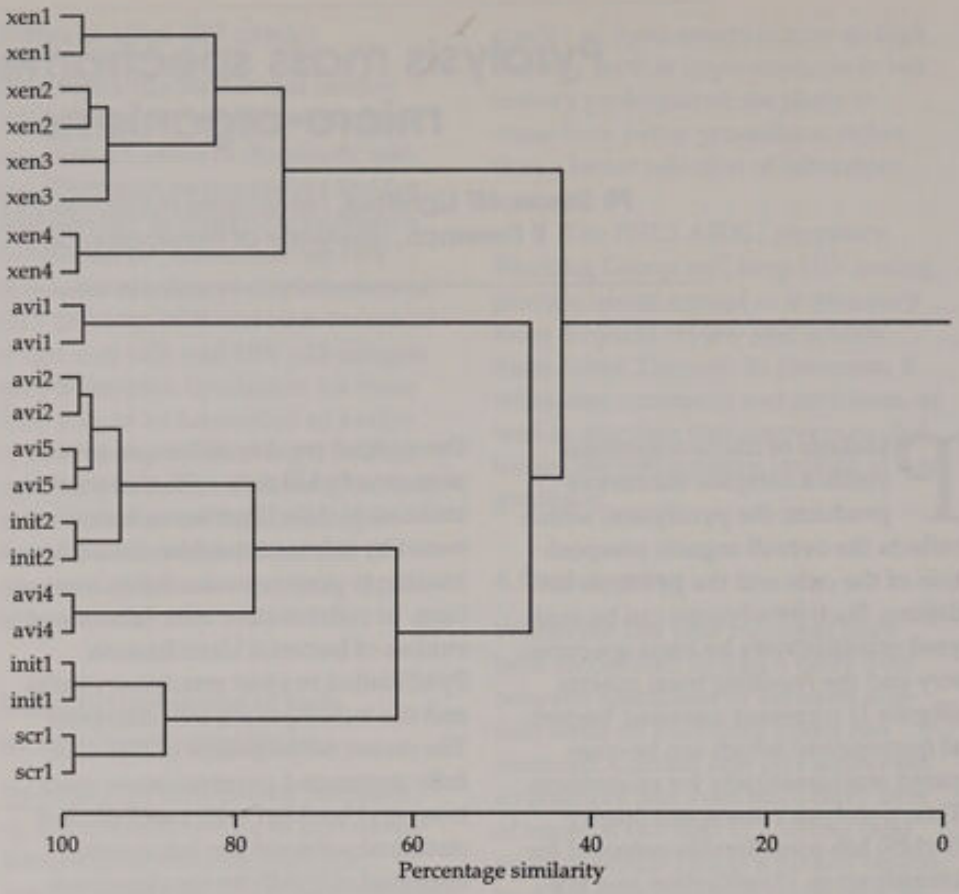


*Figure 2* An example of a similarity dendrogram from a PyMS analysis, showing percentage similarities between 4 isolate of *Mycobacterium xenopi*, 5 isolates of *M.avium*, 2 isolates of *M.intracellulare* and 1 isolate of *M Scrofulaceum*. Two subcultures of each isolate have been pyrolysed and the clustering method was UPGMA.

to principal component (PC) and canonical variate (CV) analyses to maximise discrimination between the groups. The resulting table of Mahalanobis distances may be used to produce a similarity dendrogram (Figure 2) using the unweighted pair group method with averages (UPGMA). The results of the combined PCCV analyses may also be displayed on ordination diagrams (Figure 3). Both outputs are then inspected to assess the relatedness of the groups pyrolysed, which may be inferred from the relative proximity of the PyMS-derived datapoints. Data from any isolate shown to be distinct on PyMS analysis can be edited from the dataset, which can be re-analysed to further investigate the relationships between the remaining isolates. The end-point of the analysis is a number of isolates in which there is as much difference between the duplicate subcultures of a single isolate as there is between subcultures of two different isolates. These are indistinguishable by PyMS and may be considered to be a single strain.[7,8]

Our early work concentrated on the application of PyMS to epidemiological studies of outbreaks of infection. PyMS is not a typing method *per se* because it does not assign a permanent type designation to the examined organisms, but it has

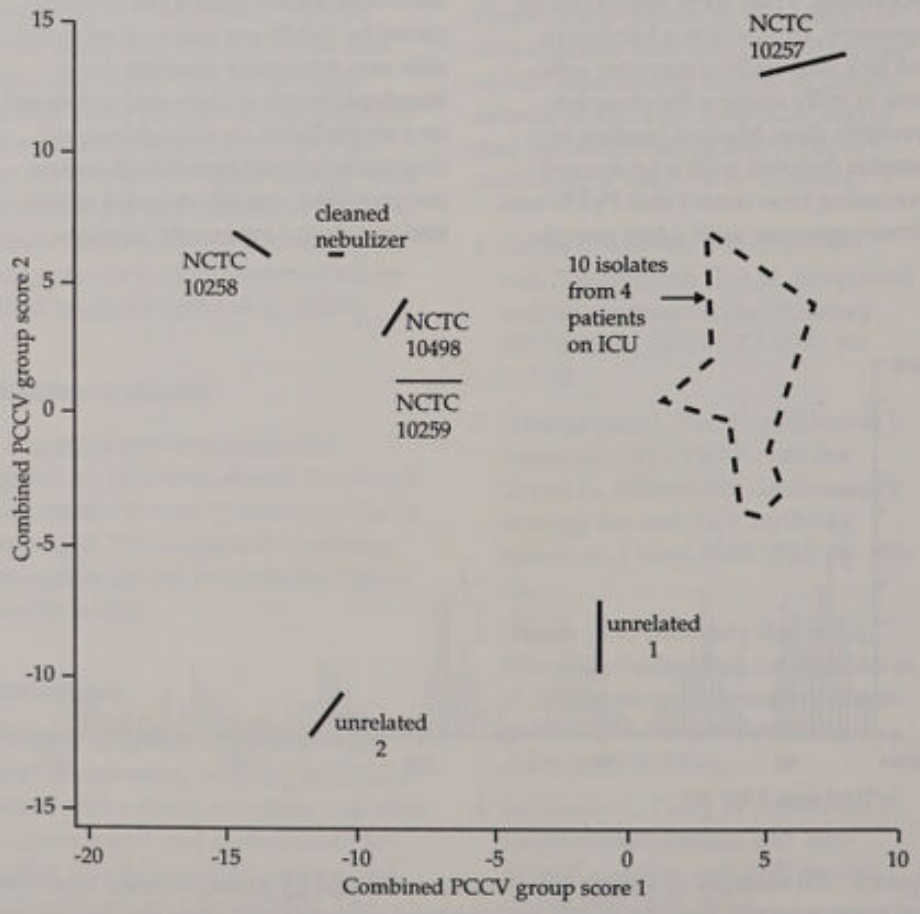


*Figure 3* An example of a PyMS-derived ordination diagram for spectral data on duplicate subcultures of 17 isolates of *Xanthomonas maltophilia*. The axes represent the first two canonical discriminant functions. Results of triplicate analyses on the same subculture have been joined together.

proved to be a quick and reliable method of inter-strain comparison for many of the species which commonly cause outbreaks. It has discriminated strains as accurately as conventional typing schemes[9, 10] and in some instances it has differentiated isolates beyond the level of routine typing systems.[11, 12] The results of PyMS analysis have been in accord with sophisticated typing based on genetic studies such as restriction fragment length polymorphism (RFLP).[9, 13] The PyMS analysis of isolates from outbreaks of infection due to species of micro-organisms for which there is no existing typing scheme have agreed with the epidemiological data subsequently available.[7, 14]

PyMS inter-strain comparison detects closely related groups of isolates from within large collections of similar organisms. This is essential for the detection of an outbreak, may indicate a likely source and facilitates the institution of effective control measures.[8, 12] The ability of PyMS to differentiate an epidemic strain from sporadic, epidemiologically unrelated isolates of the same species should enable the costly and time-consuming sophisticated typing methods of the reference laboratories to be concentrated more effectively.

PyMS studies may also establish that there is no point-source for a particular outbreak of infection, thus indicating that alternative causes must be sought and that different measures will be required to control the outbreak.[5]

The capacity of PyMS to compare many isolates within a single batch quickly and accurately may also be useful for strain identification under specific circumstances. Pyrolysis studies of mycobacteria have yielded promising results. The technique can differentiate strains within the *Mycobacterium tuberculosis* complex[15,16] and has been shown to distinguish strains of *M.xenopi* from those belonging to the *M.avium-intracellulare* complex. Pyrolysis mass spectrometry of primary isolates of slow-growing mycobacteria may enable rapid identification provided that appropriately-grown cultures of the various species with equivalent growth characteristics are available for comparative purposes.

The discriminatory ability of PyMS has been explored by examination of very closely related strains of bacteria. The technique can readily distinguish isolates of *Staphylococcus aureus* which differ only in the presence or absence of a single plasmid, or in a single point mutation on the genome conferring resistance to fusidic acid.[17] It has been assumed that PyMS is a phenotypic typing system, and hence it could be argued that in the above examples PyMS detected the products of the altered genetic material and not solely the minute genetic differences themselves. To explore this, we have compared the PyMS analysis of whole cell samples from cultures of *S.aureus*, *S.hominis*, *S.epidermidis* and *Streptococcus pyogenes* with that of purified DNA extracted from the same organisms. In both, PyMS analysis differentiated the four organisms, thus confounding the assumption that PyMS detects only phenotypic differences. Further work is under way to explore the basis for the differentiation of the DNA extracts.[18]

We have not examined the use of PyMS for taxonomic studies but others have reported success with this technique[19-21] and it obviously merits further study.

Our PyMS work to date has largely been focused on epidemiological studies. It is important to culture the micro-organisms on a rich but bland medium which does not stress them into various phenotypes which would easily be detected as different by PyMS and might lead to the genetic similarity of the isolates of an epidemic strain being obscured. For other purposes, however, it may be highly desirable to facilitate expression of certain phenotypes prior to pyrolysis. For instance, culturing isolates on a medium known to enhance toxin production might magnify the differences between toxigenic and non-toxigenic strains, enabling PyMS to differentiate one from the other.

In conclusion, whilst many other applications of PyMS to clinical microbiology are still being investigated, it has already been established that it is a highly discriminatory method of inter-strain comparison. The speed, comparatively low running costs and versatility of PyMS make it ideal for the initial screening of suspected outbreaks of infection, allowing more expensive and time-consuming methods to be used more cost-effectively for formal typing.

Pyrolysis mass spectrometry is available at Newcastle and Cardiff Public Health Laboratories.

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# Recent changes in the occurrence of antibiotic resistance in *Salmonella* isolated in England and Wales

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## Introduction

In 1986 we reported that both drug resistance and multiple resistance (to four or more drugs) was common in *Salmonella typhimurium* isolated from humans in England and Wales in 1985 but was uncommon in other serotypes. Multiple resistance was the norm in bovine-associated phage types but was rare in those associated with poultry. It was therefore concluded that bovines were the primary reservoir of the most common phage types of multiresistant *S. typhimurium* found in humans.[1] We now report the incidence of resistance in salmonellas isolated from humans and food animals in England and Wales in 1990, with particular emphasis on multiple resistance.

## Incidence of resistance

Since 1988 *S. enteritidis* has replaced *S. typhimurium* as the most common serotype in humans; in 1990, of 29 801 salmonellas referred to the Division of Enteric Pathogens (DEP), the most common serotypes were *S. enteritidis* (63%), *S. typhimurium* (18%) and *S. virchow* (4%). The remaining 15% of isolates were comprised of strains of a further 211 serotypes. None of these accounted for more than 1% of the total and they were not of major epidemiological importance.

Both resistance and multiple resistance have remained uncommon in *S. enteritidis* but have increased in *S. typhimurium* (Table 1). In 1985 multiple resistance was uncommon in *S. virchow* and we therefore did not comment on this serotype. However, in 1990, 75% of isolates of *S. virchow* were drug-resistant and 11% multiresistant.

Table 1 **Drug resistance in *S. enteritidis*, *S. typhimurium* and *S. virchow* isolated from humans in England and Wales in 1990**

| Serotype | Number | % Sensitive | % Resistant to 1 | 2 | 3 | 4+ drugs |
|---|---|---|---|---|---|---|
| *S. enteritidis* | 17 794* | 89 | 5 | 2 | 3 | 1 |
| *S. typhimurium* | 5 451 | 47 | 14 | 8 | 13 | 18 |
| *S. virchow* | 1 216 | 25 | 52 | 6 | 6 | 11 |

* 17 794 of 18 840 strains received were tested

Table 2 **Resistance to individual drugs in *S. enteritidis*, *S. typhimurium* and *S. virchow* isolated from humans in 1990**

| Antibiotic | % resistant *S. enteritidis* (n = 17 794) | *S. typhimurium* (n = 5 451) | *S. virchow* (n = 1 216) |
|---|---|---|---|
| Ampicillin (A) | 4 | 17 | 9 |
| Chloramphenicol (C) | <1 | 6 | 5 |
| Gentamicin (G) | <<1 | 1 | 1 |
| Kanamycin (K) | <<1 | 2 | 4 |
| Streptomycin (S) | 3 | 19 | 8 |
| Sulphonamides (Su) | 2 | 40 | 13 |
| Tetracyclines (T) | 2 | 45 | 10 |
| Trimethoprim (Tm) | 1 | 21 | 12 |
| Furazolidone (Fu) | 6 | 1 | 71 |
| Nalidixic Acid (Nx) | <1 | <1 | 3 |

## Resistance to individual drugs

Table 2 shows that, for *S. enteritidis*, the only resistances of significance were those of ampicillin and high level furazolidone (MIC: >20mg/L). In *S. typhimurium* the most common resistances were tetracyclines (T), sulphonamides (Su), trimethoprim (Tm), streptomycin (S) and ampicillin (A) and 6% of strains were resistant to chloramphenicol (C). In contrast to *S. enteritidis*, only 1% of isolates of this serotype were resistant to furazolidone. Of particular note in *S. virchow* was the high incidence of resistance to furazolidone (71%). Other resistances of significance were ampicillin (9%), chloramphenicol (5%) and trimethoprim (12%).

## Resistant phage types

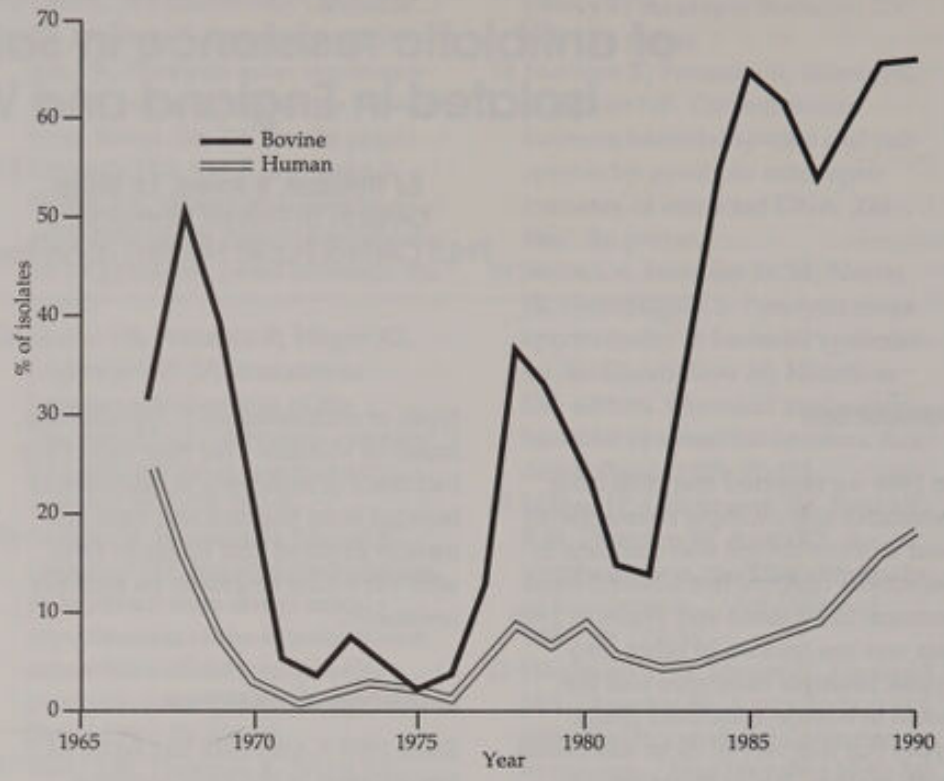
In 1990 only 1% of isolates of *S. enteritidis* were multiresistant. However,

amongst the few multiresistant strains the most common R-types were ASTFu and CSSuTTm. Strains of R-type ASTFu belonged to a drug-resistant clone of phage type 24 (PT24) which has emerged since 1987. From 1987 to 1990 there was a substantial increase in a clone of PT24 of R-type AST in humans. Genetic studies demonstrated that this clone had been derived from a drug-sensitive progenitor strain of PT4 following the acquisition of an Inc N plasmid coding for AST and for resistance to some of the *S. enteritidis* typing phages.[2] Strains of PT24 ASTFu belonged to this clone and had acquired additional chromosomally-mediated resistance to furazolidone. In PT4, although multiple resistance was extremely rare, the few strains of R-type CSSuTTm belonged to this phage type and were associated with an outbreak in the City of London related to a sandwich bar.

Since 1985 multiple resistance has increased significantly in *S. typhimurium* (Figure) and in 1990 18% of isolates were multiresistant with the most common R-types being ASSuT and ACSSuTTm. The majority of strains of R-type ACSSuTTm belong to a clone of PT204c which appeared in cattle in 1983 and which has subsequently become the predominant PT204c clone. Genetic studies demonstrated that PT204c of R-type ACSSuTTm did not carry a 36MDa plasmid coding for AK,[3] present in epidemic isolates DT204c of R-type ACKSSuTTm.[4] However, in the clone of R-type ACSSuTTm, although the AK plasmid had been lost, resistance to ampicillin had been retained as a result of the internal tranposition of the ampicillin resistance genes onto other plasmids in the strain.[3]

The majority of strains of R-type ASSuT belong to PT193. PT193 ASSuT appeared in bovines in 1985 and although increasing in incidence in bovines, has subsequently been associated with pigs and in 1990 with poultry. Since 1985 PT193 ASSuT has increased in incidence in humans to such an extent that by 1988 it had become the most common multiresistant phage type.[5] In PT193 R-type ASSuT the complete spectrum of resistance is encoded by a conjugative

*Figure* Multiple drug resistance
*S. typhimurium*, England and Wales




plasmid of approximately 80 MDa (EJ Threlfall, H Chart, B Rowe, unpublished observations).

In *S. virchow* the most common R-types in multiresistant strains isolated in 1990 were those of ACSSuTTmFu and CSSuTTmFu. The phage type in which multiple resistance was most common was PT19, a phage type associated with poultry meat imported from France.[5]

## Drug resistance in strains from food animals

### *Isolates from cattle*

*S. typhimurium* remains the serotype most frequently isolated from calves in England and Wales[6] and since 1985 the incidence of multiple resistance has remained essentially unchanged (Table 3, Figure). However, the incidence of resistance to chloramphenicol, gentamicin and trimethoprim has fallen and of particular note is the dramatic decline in resistance to kanamycin, from 53% in 1985 to only 2% in 1990 (Table 3). The most important factors contributing to these changes have been a decline in the overall incidence of *S. typhimurium* PT204c in bovines, from 62% of isolates in 1985 to 52% in 1990, and a marked decline in the isolation of strains of R-types ACKSSuTTmand ACGKSSuTTm (G = gentamicin). Unfortunately, since 1985 there has been an upsurge in isolations of PT204c of R-type ACSSuTTm and in

Table 3 **Drug resistance in *S. typhimurium* isolated from bovines in 1985 and 1990**

| Year | Total | %DR | %MR | % Resistant to A | C | G | K | T | Tm | Fu |
|---|---|---|---|---|---|---|---|---|---|---|
| 1985 | 1 638 | 77 | 65 | 64 | 64 | 16 | 53 | 73 | 65 | 1 |
| 1990 | 1 178 | 79 | 66 | 62 | 45 | 8 | 2 | 76 | 53 | 1 |

Resistance symbols: see Table 2
DR = resistant to 1 or more antimicrobial
MR = resistant to 4 or more antimicrobials

1990, 77% of PT204c from cattle were of this R-type. Furthermore, isolations of PT193 ASSuT from cattle have increased from 0.1% in 1985 to 9% in 1990.

### *Isolates from poultry*

Although resistance has remained uncommon in salmonellas isolated from poultry, of particular note has been the isolation of a small number of strains of *S. enteritidis* PT24 of R-types AST and ASTFu from chickens[2] and the isolation in 1989 and 1990 of strains of *S. typhimurium* of R-types ACGKSSuTTm, ACKSSuTTm and ACSSuTTm from chickens and turkeys.[7,8] The latter strains either belonged to PT204c[7] or were untypable.[8]

## Conclusions

In 1986 we concluded that legislation introduced in 1971 to control the appearance in food animals of multiply-resistant salmonellas appeared to have failed and stressed that a reduction in antibiotic usage coupled with improved hygiene in the husbandry and transportation of calves was essential to effect an improvement. In 1984 the British Veterinary Association produced guidelines aimed primarily at reducing the number of occasions a calf may be sent to public market during the first 56 days of life, when the animal is at most risk of salmonella infection.[9] It now appears that changes in marketing techniques may have had some impact and resulted in an overall decline in the incidence of *S. typhimurium* in calves.[6,10] However, results presented above demonstrate that the incidence of multiple resistance in bovine *S. typhimurium* remains at above 60%, multiresistant strains of PT204c still comprise over 50% of isolates and strains of PT193 ASSuT are increasing in incidence.

These findings confirm the need to continue surveillance of antimicrobial resistance in salmonellas from humans and food animals and demonstrate that ongoing reappraisal of the use of such agents in animal husbandry is called for. It is important to realise that the use of antimicrobials in countries other than the UK can lead to the appearance of multiresistant strains in the UK, as demonstrated by the appearance of multiresistance in phage types of *S. virchow* associated with poultry meat imported from France. In this respect, and also in relation to the use of quinolone drugs in animal husbandry in some European countries,[11] it would appear that a concerted international approach to control antibiotic usage is required.

Ongoing surveillance activities in the DEP such as those described above aim to provide information which can assist in the formulation of policies on the use of antibiotics in human and veterinary medicine. With respect to human infections, such information may also be useful as a guide to clinicians in the treatment of invasive salmonellosis.

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# The virology of the water cycle

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## Introduction

In the natural water cycle, rainwater accumulates to form streams, rivers and lakes. The flow is into the sea or by seepage into deep rock. The surface layer of the sea evaporates to water vapour which in turn condenses to raindrops. Upon this basic cycle man has imposed a complex network of water storage, abstraction, usage and discharge. Abstraction is from rivers, lakes, rock aquifers and, in arid countries, the sea. After domestic, agricultural or industrial use the sewage and wastewater has to be disposed of or treated and recycled.

Human viruses enter the network in faeces, blood and urine disposed of as sewage. Bloodborne viruses such as hepatitis B virus (HBV) and HIV will be present in blood and urine from infected carriers. These viruses can survive for days in liquids though the harsh conditions of sewage would afford the particles little protection. HBV is usually present in blood in higher titre than HIV but in both cases survival time would be limited.[1] In contrast, viruses that replicate in the gut and are shed in faeces are surrounded by protective organic debris. Some of these viruses are able to withstand environmental conditions and spread readily from person to person by the faecal-oral route.[2] All viruses are obligate intracellular parasites so replication does not take place outside the living host.

The viruses of the gut comprise several unrelated groups. Significant damage resulting in gastroenteritis occurs when the following viruses replicate in the epithelium covering the villi of the gut: rotavirus, astrovirus, adenovirus 40, 41, SRSV(Norwalk–like viruses), and calicivirus.

These viruses cause gastoenteritis in a variety of patterns. Rotavirus is a major cause of childhood diarrhoea with a distinct seasonality. Although endemic, the peak incidence in the UK occurs between December and March; adult disease is unusual except for the elderly in residential care. Astrovirus and calicivirus infections occur on a large scale in early childhood and include many asymptomatic infections,[3] while adenovirus serotypes 40 and 41 are associated with diarrhoea in babies under one year.[4] The SRSV group, by contrast, cause outbreaks of diarrhoea and vomiting in all age groups.

Poliovirus, coxsackievirus A and B and echovirus make up the enterovirus group and may cause fever, malaise, myalgia, meningitis and, rarely, paralysis. These symptoms occur when the viruses infect tissue outside the gut though the majority of infections, especially amongst children, are asymptomatic. Coxsackievirus B and echovirus serotypes circulate in the UK population in largest numbers during late summer and autumn. The predominant serotype varies from year to year though the symptoms of a flu–like illness are common to many serotypes. Diarrhoea only occurs occasionally and is usually part of a generalised illness.[5,6]

The cultivable adenoviruses infect eyes and the respiratory tract causing conjunctivitis, fever, malaise, coryza and rashes. Replication in the gut by adenovirus serotypes such as 1 to 7 does not result in gastroenteritis.

The liver is infected by hepatitis A virus and by the recently described hepatitis E virus, two other viruses that spread by the faecal–oral route.[7] HAV is endemic in the UK at a low prevalence level, with major increases in activity every 9–10 years.[8] Infection is often subclinical in children. In areas of the world with poor quality water supplies it is very common. HEV has been linked with serious outbreaks of hepatitis in the Indian subcontinent and the Far East. HAV and HEV are shed in faecal material. Other enteric hepatitis-associated viruses may yet remain to be identified.

Reovirus and coronaviruses replicate in the gut and may be present in faeces but have not been associated with any specific disease.

## Water processing to concentrate virus

In the UK and the USA the most widely used concentration method is based on virus adsorption to a filter matrix. This is followed by elution from the filter and flocculation and deposition of protein. A variation of this method is used for soils and sludge. Adsorption to and elution from the inorganic particles of the sample is achieved by pH changes using beef extract or skimmed milk diluents. In general, a high concentration of organic material will reduce the efficiency and reproducibility of a method. Other methods in use include ultrafiltration, magnatite beads, glass powder and PEG. Methods developed for enteroviruses may not be suitable for rotavirus concentration.[9]

## Detection

Originally only the cultivable enteroviruses were isolated from water. The BGM cell line is widely used as it supports the growth of a wide range of enterovirus serotypes.[10] The suspended cell plaque assay allows a direct estimation of the virus particle count (plaque–forming units: pfu) in three days. As used in the UK only poliovirus and coxsackievirus B are detected. For the detection of these

viruses from water concentrates it is an efficient, reproducible system that fulfils the requirements of the EC bathing water directive. If a wider range of enteroviruses, eg echovirus, are to be identified this plaque system must be adapted or the cells must be maintained under a liquid medium for 7–10 days.[11]

Rotavirus can now be identified from water by culture of samples on sensitive cell lines (LLCMK2 or MA104). The rotaviruses undergo an incomplete replication cycle and viral antigens are then detected using immunofluorescent or immunoperoxidase stains. The electron microscope and ELISA detection techniques commonly used on faecal material are not sufficiently sensitive to detect the small numbers of particles in water samples.

Human enterovirus and rotavirus are the most likely viruses to be detected by these systems but animal viruses may also be present in water. Bovine enteroviruses are readily cultured on BGM cells (author's unpublished observations). Isolates of bovine rotaviruses cannot be distinguished from human ones without specific tests.

Hepatitis A virus, the SRSVs and the other non–cultivable viruses are difficult to detect in faecal material. Viral gene molecular methodologies have not yet produced practical procedures for use with water samples. A major difficulty is the non–specific interference on test systems from the natural components of water such as humic and fulvic acid.

## Sewage treatment

The viruses found in raw sewage are those currently circulating in the community.[12] Viruses are partially removed from the liquid phase of sewage by the biological and sedimentation processes of sewage treatment.[13] Viruses trapped in debris will be sedimented and removed to the sludge portion of sewage. Thus, treated effluent only contains 0–50% of detectable viruses present in raw sewage. The solids of sewage are usually treated by anaerobic digestion during which microbial action will further reduce virus numbers. However, enteric viruses will survive the temperature and the other conditions within digesters.

Rivers receive treated effluent while the sea receives discharges of raw sewage, treated effluent and concentrated sludge. Viruses survive and persist for months at low temperature, high suspended solid content and low biological activity.[14, 15] UV light does denature viruses but penetration through water is limited by depth and the presence of organic material. Virus inactivation will occur by breakdown of protein coat and nucleic acid and by predation.

## Drinking water

During treatment to produce potable water the organic material in the source water is removed by a series of coaggulation and flocculation steps. Viruses, often attached to this debris, should be removed by this process.[16] The finished water is chlorinated to inactivate viruses and bacteria and to prevent regrowth of bacteria in the distribution system.

SRSVs, rotaviruses and hepatitis viruses have been associated with disease when drinking water systems[17] or source water[18] has become contaminated.

## Recreational water

Sewage, sea and fresh water will contain the same range of virus types.[19] Certain serotypes are rarely identified, such as poliovirus 3, even though in the UK this virus is constantly seeded into the human population through the live vaccine programme. Poliovirus serotypes in sewage and therefore receiving water are vaccine-associated.[12] Ingestion of these viruses poses no risk to fully vaccinated individuals.

An EC directive has initiated sampling of seawater at bathing beaches during the summer season.[20] In 1990, 813 samples and in 1991, 990 samples were tested for enterovirus content from nine National Rivers Authority regions. Usually two 10-litre samples were taken from each beach. No virus was found in two thirds of the samples and over 91% contained fewer than 5 pfu/10 litres. Twenty-eight samples in 1990 (3%) and 39 in 1991 (4%) contained more than 10 pfu/10 litres. High numbers in one 10-litre sample did not necessarily mean that the other 10-litre sample would contain virus at a high level or any virus at all. For both years it appears that when samples were taken early in the season (May-July), more contained detectable virus - 39% in 1990 and 42% in 1991. Late in the season (August-September) the figures were 26% in 1990 and 19% in 1991. This is unexpected as the peak enterovirus isolation rate from the population is during late summer and autumn.

Does this monitoring provide useful virological information? The results demonstrate that the detection of virus is sporadic and the counts low. The virus types detected are limited and because of differing epidemiologies cannot be used as indicators for other virus groups. Sample size, sample frequency and the inefficiency of the concentration processes determine that the detection rate is low. It would therefore seem that more generalised monitoring is not at present justified.

Is the EC bathing water directive standard of 0 pfu/10 litres a realistic level? The sporadic nature of the positive results would suggest that complete absence of virus in all bathing water is impossible to achieve while untreated sewage is discharged to sea. A more practical standard might be 'not more than 5 pfu/10 litres'. Can any standard be linked to a definite health risk? Theoretically 1 pfu is capable of initiating infection in a susceptible host and the number of viruses detected in bathing waters are minimum values. But do 5 pfu/10 litres of enterovirus – ie 1 pfu/2 litres of water - represent a health risk?

Recently several epidemiological studies have attempted to quantify the risk of disease from bathing.[21] Symptoms involving eyes, ears and throat, and some diarrhoea, have been reported at low but significant levels, and the incidence of illness seems to be related to exposure to water of poor microbiological quality as

judged by the level of bacterial indicators. No single bacterial indicator correlates with enterovirus presence although several together, ie faecal coliform, faecal streptococcus and bacteriophage, may.[22] Viruses are often suggested as the cause of these illnesses through no specific virus type has been clinically associated. Eye, ear and throat symptoms are not commonly linked with enteroviruses or the respiratory adenoviruses in the absence of more generalised clinical manifestations. Diarrhoea is not caused by enteroviruses and adenovirus serotypes 40 and 41 and astrovirus have only been significantly linked with diarrhoea in babies under one year.

Rotavirus infection and illness are minimal and sporadic during the summer months. SRSVs (Norwalk virus) tend to occur as community outbreaks. The epidemiology of astrovirus and calicivirus does not suggest increased detection rates during summer months or a relationship with bathing. None of these viruses fits neatly into the role of causative agent(s). As yet unidentified virus types may be involved, but perhaps other microbes, chemicals or irritants are more important.

### Shellfish

Bivalve molluscs such as clams, oysters and mussels are harvested from seawater close to shore and from estuaries. Viruses may be concentrated in their flesh if the surrounding water contained sewage. Depuration, in which the shellfish are cleansed in UV-treated water, does not remove viruses.[23] When appropriate, cooking will inactivate SRSV and HAV. Outbreaks of disease have been caused by these viruses but not by rotavirus. Detection of any virus from shellfish is very difficult and testing is not practical.

### The future

The newer laboratory techniques of PCR, gene probe and bioluminescence may allow the detection of SRSV, HAV and rotavirus from water, although many practical problems remain before testing can be routine. The extra information, though of interest, is not essential for the adequate treatment of drinking and wastewater.

In the near future treatment of sewage will be required before coastal wastewater can be discharged to sea. Dumping of sewage sludge to sea must cease by 1998. Measures to reduce suspended solids in final effluent are being introduced. Studies on the UV treatment and disinfection of effluent are also under way. All these measures should reduce the number of human viruses discharged to fresh and seawater. Water should be viewed as a valuable resource which must be protected and conserved.

Drinking water treatments include chlorination to eliminate any viruses which may be present. These treatments must now include procedures to deal with the real threat of Cryptosporidium contamination. The facilities in the UK can be expected to be made sufficient to ensure that water is not a route of virus transmission.

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# Water Virology Discussion Group

The UK Water Virology Discussion Group has existed for over 10 years. Founder members were from four Water Authority virus laboratories, Surrey University and Reading PHL. Over the last two years interest in the subject has expanded so that at our meeting in May this year over 20 people from 14 organisations were present. We remain an informal discussion group actively involved in various aspects of water virology. Work is now done by five water companies, a private firm, the National Rivers Authority, the Water Research Centre, MAFF and two PHLs.

The first stage of a water virology quality assurance pilot study was reviewed at the last meeting. Methods for processing water and for detecting and counting viruses are similar in the various laboratories. Distributions of virus have been tested and results compared. Interesting differences in counts have been highlighted. Further development in this study is under way.

Discussions also included EC bathing water monitoring and disinfection studies on sewage and effluent. It was stated that chlorination of sewage and effluent is unlikely to become widespread. Many general aspects of sewage treatment and disposal are now being re–assessed for reasons of economy, conservation, environmental damage and public pressure.

The next meeting of the group is planned for November.

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# Bacteriological examination of shellfish

Ministry of Agriculture, Fisheries and Food, Department of Health and Public Health Laboratory Service Working Group*

## Introduction

The Council of the European Communities has published a Council Directive (91/492/EEC) which sets out the conditions for the production and marketing of live bivalve molluscs.[1] This directive provides for an end product standard and the classification of shellfish harvesting areas into categories according to the degree of contamination with faecal indicator bacteria (*Escherichia coli*) in samples of shellfish flesh (Table 1).

To ensure standardisation of methodology, the Ministry of Agriculture, Fisheries and Food, the Department of Health and the Public Health Laboratory Service established a small working group to draft proposed methods. The Working Group's proposals were then circulated to the following interested bodies for consultation:

- National Rivers Authority
- Department of Agriculture for Northern Ireland
- The Scottish Office
- Welsh Office
- The Shellfish Association of Great Britain
- The Association of Public Analysts
- The Fishmongers Company
- Scottish River Purification Boards
- PHLS laboratories

This article details the laboratory methodologies for examination of shellfish for *Escherichia coli* (classification of harvesting areas and end product standard) and *Salmonella* sp. (end product standard only) which were drawn up following the consultation process. All PHLS laboratories are requested to use these techniques for isolation of *E. coli* and *Salmonella* sp. from shellfish so that results can be evaluated and compared nationally.

## Recommended methods

The choice of appropriate methods is restricted by the requirement in the directive to use a most probable number (MPN) test which has a range of sensitivity down to 230 *E. coli* per 100 grams of shellfish flesh and specificity for *E. coli* or faecal coliforms. Alternative bacteriological procedures shown to be of at least equivalent accuracy may also be used.

For *E.coli*, the two-stage most probable numbers (MPN) method of West and Coleman using minerals modified glutamate broth (MMG) in a series of 5 tubes per dilution is the recommended method.[2] The use of MMG in the first stage allows the recovery of sublethally injured cells of *E.coli*. Double strength MMG in the first series of dilutions and the incorporation of 4-methylumbelliferyl B-D glucuronide (MUG) in the peptone water for *E.coli* confirmation are sensible variations of the original technique.

This method is used for the identification of *E.coli*, not coliforms. Directive 91/492 does not give *E.coli* levels for categories C and D (Table 1). Therefore, where samples give results indicating areas of high pollution levels (eg >4,600 *E.coli*/100g shellfish flesh), second stage tests can be limited to gas production at 44°C to identify only faecal coliforms.

The test for *Salmonella* sp. is only required for shellfish intended for immediate consumption (ie the end product standard). A two-stage method has been successfully used to isolate salmonella in other food investigations. The recommended method for shellfish consists of buffered peptone water/Rappaport enrichment in conjunction with the xylose lysine desoxycholate (deoxycholate) (XLD) and brilliant green agar selective plating technique. In certain circumstances tests for the presence of *S.typhi* may also be required. An additional, more specific, method for the isolation of *S.typhi* is described in this article.

The EC directive acknowledges that methodologies can be improved and that there is a need to evaluate alternatives. To this end, collaboration between laboratories is encouraged. In particular, it is suggested that investigations should be directed to improving direct detection of *E.coli* using MUG in new media formulations that could overcome any potential false positive reactions. Temperature control at the resuscitation stage and both impedance and conductance techniques are also areas which require further evaluation.

Any new technique must be tested in parallel with

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**Table 1 Classification of harvesting areas**

| | *E.coli*/100g | Faecal coliforms/100g | MPN table | |
|---|---|---|---|---|
| Category A | <230 | <300 | 1 | May go for direct consumption |
| Category B | <4 600 | <6 000 | 1,2 | Must be depurated, heat treated or relayed to meet Category A |
| Category C | (<46 000) | <60 000 | 2,3 | Must be relayed for long period (2 months) to meet Category A or B (may also be heat treated by approved method) |
| Category D | (>46 000) | >60 000 | 3 | Prohibited |

( ) figures not included in EEC Regulations.

the currently recommended MPN method in order to demonstrate at least the same level of specificity and sensitivity before being acceptable as a replacement.

## 1 EXAMINATION OF SHELLFISH FOR *ESCHERICHIA COLI*

### *1.1 Sample size*

| | |
|---|---|
| Oysters and clams | 10 - 15 |
| Mussels | 15 - 30 |
| Cockles | 30 - 50 |

### *1.2 Sample transport*

Use a cool box with freezer packs to keep at near 4°C. Samples should be delivered to the laboratory as soon as possible. Difficulties may occur in the timing of transport to fit in with the laboratory schedule. The maximum time from collection to the commencement of tests should be 24 hours provided that the sample is stored at 4°C during that time. **Samples should not be frozen** and freezer packs should not be in direct contact with samples.

### *1.3 Sample preparation*

1 Gloves should be worn during sample preparation.

2 Discard any gaping shellfish and those showing obvious signs of damage.

3 Select **at least 10** oysters or 15 mussels or cockles.

4 Scrub shellfish clean under running tap water of potable standard.

5 Dry using clean paper towels.

6 Open shellfish using a sterile shucking knife (flamed and cooled) by the following methods.

#### *1.3.1 Oysters/clams*

Insert the knife between the two shells towards the hinge end of the animal. Push the knife further into the animal and prise open the upper shell, allowing any liquor to drain into a sterile weighed bag or beaker. Push the blade through the animal and sever the muscle attachments by slicing across the animal. Remove the upper shell and scrape the contents of the lower shell into the bag or beaker. Repeat for 10 oysters/clams and collect in the same bag or beaker.

#### *1.3.2 Mussels/cockles*

Insert the knife in between the shells of the animal through the byssal opening and separate the shells with a twisting motion of the knife. Collect the liquor from the animal in a weighed sterile bag or beaker. Cut the muscle between the two shells and scrape the contents into the sterile bag or beaker. Repeat for at least 15 mussels or cockles and collect in the same bag or beaker.

### *1.4 Homogenisation and dilution of sample*

#### *1.4.1 Using stomacher*

Place the bag containing shellfish meat and liquor inside two more bags; this will prevent small pieces of shell from puncturing the bag. Remove excess air and place in the stomacher. Operate stomacher for 2-3 minutes.

Remove 50g* of homogenate to another stomacher bag. From a measured volume of 450ml sterile dilution fluid (0.1% Peptone) add approximately 100ml to the 50g sample. Homogenise in stomacher for 2-3 minutes and add to remainder of the dilution fluid. This gives the master $10^{-1}$ dilution.

From the $10^{-1}$ dilution prepare a $10^{-2}$ dilution by taking 10ml of the $10^{-1}$ to 90ml dilution fluid.

*Another 25g of homogenate may be removed at this stage for Salmonella examination.

#### *1.4.2 Using blender (homogeniser)*

Weigh beaker containing shellfish meat and liquor to the nearest gram, subtract weight of beaker and add two parts by weight of sterile dilution fluid. Homogenise mixture at high speed (approximately 12,000 rev/min) for a total of 60 seconds (4 sessions of 15 seconds blending with 15-second intervals). Stand for 30 seconds, swirl briefly and transfer 30ml of homogenate to a measured volume of 70ml of sterile dilution fluid. This gives the master $10^{-1}$ dilution.

From the $10^{-1}$ dilution prepare a $10^{-2}$ dilution by taking 10ml of the $10^{-1}$ to 90ml dilution fluid. Further dilutions, eg $10^{-3}$ (0.001g), $10^{-4}$ (0.0001g) may be needed for categories C and D harvesting areas.

### 1.5 Examination methods

#### 1.5.1 Medium

**Minerals modified glutamate medium (MMG)**
Base Oxoid CM607 plus sodium glutamate L124 and ammonium chloride. This medium is prepared as double strength and single strength (see box, facing page) in volumes of 10ml in bottles containing an inverted Durham tube to detect gas production.

#### 1.5.2 First stage tests

**1.5.2.1 Inoculation**
1 10ml of $10^{-1}$ to each of 5 tubes of double strength MMG
Each tube contains equivalent to **1g tissue.**
2 1ml of $10^{-1}$ to 5 tubes of single strength MMG.
Each tube contains equivalent to **0.1g tissue.**
3 1ml of $10^{-2}$ to 5 tubes of single strength MMG.
Each tube contains equivalent to **0.01g tissue.**

**1.5.2.2 Incubation**
Incubate at 37°C (± 1°C) for 18-24 hours in a waterbath or incubator. Examine for gas production (media will turn yellow with acid production).

If bottles are negative for gas reincubate for a further 24 hours at 37°C and then re-examine for gas. Absence of gas constitutes a negative result for *E.coli*.

#### 1.5.3 Second stage tests

**1.5.3.1**
Tubes showing gas production are examined as follows. Subculture bottles using a loop to

a) Brilliant green bile broth tubes of 5ml volume (BGBB)

b) 1% Tryptone water tubes of 5ml volume (TPW). Incubate at 44°C ± 0.2°C for 24 hours using an accurately controlled water bath or incubator.

**Control cultures:**

| | | |
|---|---|---|
| NCTC10418 | *E.coli* | gas + indole + |
| NCTC9528 | *Klebsiella aerogenes* | gas - indole - |

Examine BGBB for gas production. Examine TPW for indole using Kovac's reagent.

**Results**
Gas and indole positive at 44°C=*E.coli*
Gas only positive = Negative (faecal coliform)
Gas and indole negative = Negative
Indole positive, gas negative = Negative

OR

**Method 1.5.3.2**
Subculture bottles using a loop to

a) brilliant green bile broth (BGBB) of 5ml volume

b) 1% tryptone water with 0.05% 4-methylumbelliferyl-B-D glucuronide (MUG) (TPW+MUG) of 1ml volume.

Incubate at 44°C ± 0.2°C for 24 hours using an accurately controlled waterbath or incubator. Examine BGBB for gas production. Examine TPW + MUG for B-glucuronidase activity (BGUR) using a suitable UV lamp at 366nm.

Positive = white fluorescence. This should be performed before adding Kovac's reagent.

Examine TPW + MUG for indole using Kovac's reagent. Control B glucuronidase positive and negative cultures should be inoculated into TPW + MUG with each batch of tests. Uninoculated TPW + MUG should be checked for autofluorescence as some glassware may give problems.

**Control cultures**

| | | |
|---|---|---|
| NCTC10418 | *E.coli* | B GUR positive |
| NCTC9528 | *Klebsiella aerogenes* | B GUR negative |

### 1.6 Results

Gas positive, indole positive, B GUR positive = *E. coli*
Gas positive, B GUR positive = *E. coli*
Indole positive. B GUR positive = *E. coli*
Gas positive, indole positive, B GUR negative = *E. coli*
Gas indole/B GUR negative = Negative

Other combinations are possible but should be considered negative at the present time.

### 1.7 Final results

Following completion of the 2nd stage tests for *E. coli* the combination of postive *E. coli* bottles/tubes for the dilution range can be determined by reference to the MPN tables. Results are expressed as the number of *Escherichia coli* per 100 grams of shellfish.

## 2 EXAMINATION OF SHELLFISH FOR *SALMONELLA* SP

Tests for salmonella are only required to be applied to shellfish intended for immediate human consumption (final product) and not for the classification of harvesting areas. In certain circumstances tests for the presence of *Salmonella typhi* may be required. A general method for salmonella which will recover a wide range of serotypes is given below. However, this method is not satisfactory for the specific isolation of *S.typhi* and an additional method for its isloation is detailed.

### 2.1 General Method for Salmonella

#### 2.1.1 Sample preparation

See Section 1.3.

### 2.1.2 Inoculation/homogenisation

a) **Stomacher used for *E.coli***
Remove 25g of bulk homogenate to a 250ml or larger sterile bottle. Add 225ml of buffered peptone water. Mix well. Incubate at 37°C for 18-24 hours.

b) **Blender used for *E.coli***
Weigh out a separate 25g amount in a sterile beaker. Add approximately 50ml of BPW from a 225ml bottle and homogenise at high speed for a total of 60 seconds (see Section 1.4.2). Add to remainder of PBW in the bottle and mix well. Incubate at 37°C for 18-24 hours.

### 2.1.3 Selective enrichment

Remove 0.1ml of the incubated BPW and transfer to 10ml of sterile Rappaport-Vassiliadis enrichment broth (RV). Incubate at 41 - 42°C in a water bath or incubator.

### 2.1.4 First subculture

After 18-24 hours incubation subculture the RV using a loop of 2-3mm diameter to the following whole plates inoculating to give colonial separation:

a) Xylose lysine desoxycholate (deoxycholate) agar (XLD)

b) Brilliant green agar (BGA).

Incubate the XLD and VGA plates at 37°C for 18-24 hours. Re-incubate the RV.

### 2.1.5 Second subculture

Subculture RV after 48 hours to XLD and BGA. Incubate at 37°C for 18-24 hours. Discard RV. Examine XLD/BGA plates for salmonella-like colonies after 18-24 hours incubation. Investigate salmonella-like colonies using standard biochemical and serological methods.

Alternative methods, eg impedance or conductance, require detailed evaluation before they can be used in place of the standard method for shellfish.

## 2.2 Method for Salmonella typhi

### 2.2.1 Inoculation/homogenisation

Remove 25g of bulk homogenate to a 250ml or larger sterile bottle. Add 225ml of buffered peptone water (BPW). Mix well. Incubate at 37°C for 18-24 hours.

### 2.2.2 Selective enrichment

Remove 10ml of the incubated BPW and transfer to 100ml of sterile Selenite F broth. Incubate at 37°C (± 1°C) in a waterbath or incubator.

### 2.2.3 Subculture

After a full 24 hours incubation of the Selenite F, subculture using a loop of 2-3mm diameter to the following whole plates, inoculating to give colonial separation.

a) Desoxycholate citrate agar (Hynes modification) (DCA)

b) Bismuth sulphite agar (Wilson and Blair Modified) (WB)

Incubate at 37°C for 18-24 hours. Examine DCA/WB plate for salmonella-like colonies. Re-incubate DVA/WB plates for a further 24 hours at 37°C (48 hours in

## M E D I A

***Minerals modified glutamate medium***

Oxoid CM607 Base
L124 Sodium Glutamate

*Directions*
Double strength: Dissolve 5g ammonium chloride in 1 litre of distilled water. To this add 22.7g minerals modified medium base CM607 and 12.7g of sodium glutamate L124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C.

Single strength: Dissolve 2.5g ammonium chloride in 1 litre of distilled water. To this add 11.4g of minerals modified medium base CM607 and 6.4g of sodium glutamate L124. Mix to dissolve completely. Sterilise as above.

**Other media or reagents available:**

***Brilliant Green Bile Broth***
eg Oxoid CM31
Supplied by other media companies

***Tryptone Water***
eg Oxoid CM87

***Tryptone Water with MUG***
1% Tryptone 0.05% MUG
0.5% Sodium Chloride pH 7.5

***4-methylumbelliferyl-B-D-glucuronide (MUG)***
eg Oxoid BR71 freeze-dried supplement
Sigma M-9130

***Buffered Peptone Water***
eg Oxoid CM509 pH 7.2
Supplied by other media companies

***Rappaport-Vassiliadis Enrichment Broth***
eg Oxoid CM669
Supplied by other media companies

***XLD Medium***
eg Oxoid CM469
Supplied by other media companies

***Brilliant Green Agar***
eg Oxoid CM329 (Modified)
Supplied by other media companies

***Dilution Fluid***
0.1% peptone in water
pH after sterilisation pH 7.2 +/- 0.1

***Desoxycholate Citrate Agar***
Hynes modification
Oxoid CM227 Lab M 65
Supplied by other media companies

***Bismuth Sulphite Agar***
eg Lab M13A and 13B
Supplied by other media companies

***Selenite F Broth*** (0.4%)
Oxoid CM399 with sodium biselenite L121 to give 0.4% concentration
Care must be taken with sodium biselenite (see manufacturer's hazard notes)
Supplied by other media companies

total). Re-examine the DCA/WB plates for salmonella-like colonies. Investigate salmonella-like colonies using standard biochemical and serological methods.

### Most probable number (MPN) tables[3]

These MPN tables are to be used for confirmed *Escherichia coli* positive test results. The dilution series gives an estimate of the number of organisms present rather than a count. Early publications looked at the imprecision of these estimates, but the mathematical methods used were approximate. A more recent study used exact probability methods and illustrated that the imprecision can be considerable, especially if most tubes give a positive reaction. Tabulations of 'most probable ranges' were published. They demonstrate which numbers are close contenders for the title MPN and include all counts which are at least 95% as likely as the MPN to be the correct count.

It is important to remember that the result (and any qualifying statement about its imprecision) relates to the material examined. For more information, such as an estimate of the average contamination with confidence intervals relating to the batch of shellfish, it would be necessary to take multiple samples.

These tables do not include unlikely combinations of results (eg 1g and 0.1g with negative results and 0.01g with positive results). These results should be considered suspect and indicate that the sample and test should be repeated.

**Table 2** (using 5 x 1g, 5 x 0.1g, 5 x 0.01g) will cover MPN results in Category A and most of lower Category B.

**Table 3** (using 5 x 0.1g, 5 x 0.01g, 5 x 0.001g) will cover most higher Category B and lower Category C.

**Table 4** (using 5 x 0.01g, 5 x 0.001g, 5 x 0.0001g) will cover higher Category C and Category D.

*Note* Test results which fall into Category D will not need full *E.coli* Stage 2 tests, only tests for gas at 44°C (faecal coliforms). *E.coli* figures are not included in the EEC table for harvesting areas C and D.

Results should be reported as the revised MPN number per 100 grams. An interpretation of the result in relationship to the classification of the harvesting area should **not** be made by the laboratory.

## Table 2 Probable numbers of organisms*

**Revised tables for multiple method using 5 x 1g, 5 x 0.1g, 5 x 0.01g**

Results expressed as MPN per 100 grams

| 1g | 0.1g | 0.01g | Revised MPN | |
|---|---|---|---|---|
| 0 | 0 | 0 | none detected | |
| 0 | 0 | 1 | 20 | |
| 0 | 1 | 0 | 20 | |
| 1 | 0 | 0 | 20 | |
| 1 | 0 | 1 | 40 | |
| 1 | 1 | 0 | 40 | |
| 1 | 2 | 0 | 50 | |
| 2 | 0 | 0 | 40 | |
| 2 | 0 | 1 | 50 | |
| 2 | 1 | 0 | 50 | |
| 2 | 1 | 1 | 70 | |
| 2 | 2 | 0 | 70 | |
| 2 | 3 | 0 | 110 | |
| 3 | 0 | 0 | 70 | |
| 3 | 0 | 1 | 90 | |
| 3 | 1 | 0 | 90 | |
| 3 | 1 | 1 | 130 | |
| 3 | 2 | 0 | 130 | |
| 3 | 2 | 1 | 160 | |
| 3 | 3 | 0 | 160 | |
| 4 | 0 | 0 | 110 | |
| 4 | 0 | 1 | 140 | |
| 4 | 1 | 0 | 160 | |
| 4 | 1 | 1 | 200 | Category A |
| 4 | 2 | 0 | 200 | (<230 *E.coli*) |
| 4 | 2 | 1 | 250 | Category B |
| 4 | 3 | 0 | 250 | (>230 *E.coli*) |
| 4 | 3 | 1 | 310 | (<4,600 *E.coli*) |
| 4 | 4 | 0 | 320 | |
| 4 | 4 | 1 | 380 | |
| 5 | 0 | 0 | 220 | |
| 5 | 0 | 1 | 290 | |
| 5 | 0 | 2 | 410 | |
| 5 | 1 | 0 | 310 | |
| 5 | 1 | 1 | 430 | |
| 5 | 1 | 2 | 600 | |
| 5 | 1 | 3 | 850 | |
| 5 | 2 | 0 | 500 | |
| 5 | 2 | 1 | 700 | |
| 5 | 2 | 2 | 950 | |
| 5 | 2 | 3 | 1200 | |
| 5 | 3 | 0 | 750 | |
| 5 | 3 | 1 | 1100 | |
| 5 | 3 | 2 | 1400 | |
| 5 | 3 | 3 | 1750 | |
| 5 | 3 | 4 | 2100 | |
| 5 | 4 | 0 | 1300 | |
| 5 | 4 | 1 | 1700 | |
| 5 | 4 | 2 | 2200 | |
| 5 | 4 | 3 | 2800 | |
| 5 | 4 | 4 | 3450 | |
| 5 | 5 | 0 | 2400 | Category B |
| 5 | 5 | 1 | 3500 | (<4,600 *E.coli*) |
| 5 | 5 | 2 | 5400 | Category C |
| 5 | 5 | 3 | 9100 | (>4,600 *E.coli*) |
| 5 | 5 | 4 | 16000 | (<60,000 faecal |
| 5 | 5 | 5 | >18000† | coliforms) |

* From Tillett[3]

† needs further dilutions to clarify classification.

## Table 3 Probable numbers of organisms*

**Revised tables for multiple tube method using 5 x 0.1g, 5 x 0.01g, 5 x 0.001g**

Results expressed as MPN per 100 grams

| 0.1g | 0.01g | 0.001g | Revised MPN | |
|---|---|---|---|---|
| 0 | 0 | 1 | 200 | |
| 0 | 1 | 0 | 200 | Category A |
| 1 | 0 | 0 | 200 | (<230 *E.coli*) |
| 1 | 0 | 1 | 400 | Category B |
| 1 | 1 | 0 | 400 | (<4 600 *E.coli*) |
| 1 | 2 | 0 | 500 | |
| 2 | 0 | 0 | 400 | |
| 2 | 0 | 1 | 500 | |
| 2 | 1 | 0 | 500 | |
| 2 | 1 | 1 | 700 | |
| 2 | 2 | 0 | 700 | |
| 2 | 3 | 0 | 1100 | |
| 3 | 0 | 0 | 700 | |
| 3 | 0 | 1 | 900 | |
| 3 | 1 | 0 | 900 | |
| 3 | 1 | 1 | 1300 | |
| 3 | 2 | 0 | 1300 | |
| 3 | 2 | 1 | 1600 | |
| 3 | 3 | 0 | 1600 | |
| 4 | 0 | 0 | 1100 | |
| 4 | 0 | 1 | 1400 | |
| 4 | 1 | 0 | 1600 | |
| 4 | 1 | 1 | 2000 | |
| 4 | 2 | 0 | 2000 | |
| 4 | 2 | 1 | 2500 | |
| 4 | 3 | 0 | 2500 | |
| 4 | 3 | 1 | 3100 | |
| 4 | 4 | 0 | 3200 | |
| 4 | 4 | 1 | 3800 | |
| 5 | 0 | 0 | 2200 | |
| 5 | 0 | 1 | 2900 | |
| 5 | 0 | 2 | 4100 | |
| 5 | 1 | 0 | 3100 | |
| 5 | 1 | 1 | 4300 | |
| 5 | 1 | 2 | 6000 | Category C |
| 5 | 1 | 3 | 8500 | (<60 000 faecal |
| 5 | 2 | 0 | 5000 | coliforms(>4 600 *E.coli*) |
| 5 | 2 | 1 | 7000 | |
| 5 | 2 | 2 | 9500 | |
| 5 | 2 | 3 | 12000 | |
| 5 | 3 | 0 | 7500 | |
| 5 | 3 | 1 | 11000 | |
| 5 | 3 | 2 | 14000 | |
| 5 | 3 | 3 | 17500 | |
| 5 | 3 | 4 | 21000 | |
| 5 | 4 | 0 | 13000 | |
| 5 | 4 | 1 | 17000 | |
| 5 | 4 | 2 | 22000 | |
| 5 | 4 | 3 | 28000 | |
| 5 | 4 | 4 | 34500 | |
| 5 | 5 | 0 | 24000 | |
| 5 | 5 | 1 | 35000 | |
| 5 | 5 | 2 | 54000 | Category D |
| 5 | 5 | 3 | 91000 | (>60 000 faecal |
| 5 | 5 | 4 | 160000 | coliforms) |
| 5 | 5 | 5 | >180000 | |

* From Tillett[3]

## Table 4 Probable numbers of organisms*

**Revised tables for multiple tube method using 5 x 0.01g, 5 x 0.001g, 5 x 0.0001g**

Results expressed as MPN per 100 grams

| 0.01g | 0.001g | 0.0001g | Revised MPN | |
|---|---|---|---|---|
| 0 | 0 | 1 | 2000 | |
| 0 | 1 | 0 | 2000 | |
| 1 | 0 | 0 | 2000 | |
| 1 | 0 | 1 | 4000 | Category B |
| 1 | 1 | 0 | 4000 | (<4 600 *E.coli*) |
| 1 | 2 | 0 | 5000 | Category C |
| 2 | 0 | 0 | 4000 | (<60 000 faecal |
| 2 | 0 | 1 | 5000 | coliforms) |
| 2 | 1 | 0 | 5000 | (>4 600 *E.coli*) |
| 2 | 1 | 1 | 7000 | |
| 2 | 2 | 0 | 7000 | |
| 2 | 3 | 0 | 11000 | |
| 3 | 0 | 0 | 7000 | |
| 3 | 0 | 1 | 9000 | |
| 3 | 1 | 0 | 9000 | |
| 3 | 1 | 1 | 13000 | |
| 3 | 2 | 0 | 13000 | |
| 3 | 2 | 1 | 16000 | |
| 3 | 3 | 0 | 16000 | |
| 4 | 0 | 0 | 11000 | |
| 4 | 0 | 1 | 14000 | |
| 4 | 1 | 0 | 16000 | |
| 4 | 1 | 1 | 20000 | |
| 4 | 2 | 0 | 20000 | |
| 4 | 2 | 1 | 25000 | |
| 4 | 3 | 0 | 25000 | |
| 4 | 3 | 1 | 31000 | |
| 4 | 4 | 0 | 32000 | |
| 4 | 4 | 1 | 38000 | |
| 5 | 0 | 0 | 22000 | |
| 5 | 0 | 1 | 29000 | |
| 5 | 0 | 2 | 41000 | |
| 5 | 1 | 0 | 31000 | Category C |
| 5 | 1 | 1 | 43000 | (<46 000 *E.coli*) |
| 5 | 1 | 2 | 60000 | Category D |
| 5 | 1 | 3 | 85000 | (>60 000 faecal |
| 5 | 2 | 0 | 50000 | coliforms) |
| 5 | 2 | 1 | 70000 | |
| 5 | 2 | 2 | 95000 | |
| 5 | 2 | 3 | 120000 | |
| 5 | 3 | 0 | 75000 | |
| 5 | 3 | 1 | 110000 | |
| 5 | 3 | 2 | 140000 | |
| 5 | 3 | 3 | 175000 | |
| 5 | 3 | 4 | 210000 | |
| 5 | 4 | 0 | 130000 | |
| 5 | 4 | 1 | 170000 | |
| 5 | 4 | 2 | 220000 | |
| 5 | 4 | 3 | 280000 | |
| 5 | 4 | 4 | 345000 | |
| 5 | 5 | 0 | 240000 | |
| 5 | 5 | 1 | 350000 | |
| 5 | 5 | 2 | 540000 | |
| 5 | 5 | 3 | 910000 | |
| 5 | 5 | 4 | 1600000 | |

* From Tillett[3]

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The Working Group expresses its gratitude to colleagues who offered valuable comments. Although many of the observations received have been incorporated, inevitably not all are included. It is hoped that this paper will stimulate discussion and debate on the relative merits of different methods, and contribute to the ongoing process of evaluation.

# Developments in intestinal protozoology: report of a WHO/Pan-American Health Organization (PAHO) Consultation

Mexico, 1991

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Protozoan infections of the intestine are common throughout the world. Such infections have a major impact in childhood, pregnancy, and in AIDS. There is high associated morbidity and mortality, with more than 58 million cases of childhood protozoal diarrhoea per year. The direct costs of management alone are estimated to be $150 million. There are about 100 000 deaths annually from amoebiasis. In 1984 it was calculated that 2% of all adults admitted to general hospitals in Mexico had an amoebic liver abscess, while the direct costs of amoebiasis used 1.6% of the budget of the Ministry of Health.

In the United States *Giardia* is the leading identified cause of diarrhoeal disease outbreaks associated with drinking water. It is responsible for a minimum of 4 000 hospital admissions per year, costing over $5 million. In 1989 in England and Wales, *Giardia* was the fifth and *Cryptosporidium* the fourth most commonly identified causes of gastroenteritis.[1] Although these organisms are an infrequent cause of death, there is increasing evidence that they contribute to chronic ill health, and exacerbate the effects of malnutrition.

The authors attended a meeting in Mexico organised by WHO and PAHO, where the epidemiology and molecular biology of *Entamoeba, Giardia, Cryptosporidium, Balantidium, Isospora* and the microspora were discussed. Recommendations for action to aid understanding of epidemiology and to develop control strategies were agreed. The impact on human health of infections with these organisms needs further study. The meeting acknowledged that financial and administrative assistance was needed to help scientists and physicians in developing countries work independently or in collaboration with other research groups.[2] It is hoped that distribution of the report of this meeting will encourage aid donors to fund, and research groups to carry out, further studies of diseases caused by intestinal protozoa. The transfer of technology, the training of workers, and the enhancement of facilities in supporting laboratories were recognised as priorities.

The participants at the meeting came from South and Central America, Europe (including England, Scotland and Wales) and the USA. Discussions were friendly and optimistic, and there was a feeling that the availability of new molecular biological tools for detection and characterisation of intestinal protozoa would have a major impact on epidemiological methods. A good example of this point is given by recent advances in our knowledge of *Entamoeba histolytica*. This organism, multiplying in the large intestine of man, has long been thought to be a single entity with variable pathogenic capacity. Compelling evidence now indicates that what is currently identified as *E.histolytica* actually comprises two morphologically indistinguishable species, differing genetically and in their capacity to cause disease.[3,4] One species is an invasive pathogen exhibiting varying degrees of virulence; the other is at most capable of producing superficial erosions of the colonic mucosa. The use of new tools such as DNA probes and monoclonal antibodies allows us to detect invasive *E.histolytica* readily. This new knowledge is leading to a reassessment of the epidemiology of amoebiasis. Only about 10% of the 500 million persons infected annually with *'E. histolytica'* as currently understood become symptomatic.[5] If there are two species of different pathogenic potential, the proportion of infections associated with disease may simply reflect the proportion of infections with 'invasive' *E.histolytica*, or (more likely) the relationship may be more complex.

There is a less well defined but related problem in *Giardia* infection, where several distinct genetic lineages of the organism have been recognised.[6,7] Their associations with symptomatic disease and with possible zoonotic reservoirs are still being worked out. Recent evidence on *Cryptosporidium*[8,9] indicates there may be a similar complexity. The meeting concluded that development of molecular biological tools for diagnostic and epidemiological purposes should be encouraged. Furthermore, in view of the potential significance of differences between isolates, the maintenance of a reference collection of

strains of important intestinal protozoa was recommended.

Drugs used for treatment of intestinal protozoan infections are often unpleasant to take, have adverse side effects and require repeated dosing,[10] which leads to problems of logistics and compliance. The meeting recognised an urgent need for development and evaluation of single-dose treatments in palatable formulations.

Health education is an essential adjunct to control measures for intestinal infections. Education could be as important as protection of water sources and development of safe methods of disposal and use of human waste in agriculture. The absence of breast-feeding is recognised as the single most important risk factor for diarrhoea in young children,[11] so this practice should be actively promoted. Similarly, the encouragement of simple hygienic practices, where environmental conditions allow clean water to be made available for washing hands, is thought likely to make a major impact on disease transmission.[12] In addition to public information programmes and school-based health education, training courses on important intestinal protozoa should be encouraged for primary health and day care workers, workers in diagnostic laboratories and water treatment plant operators.

It was agreed by the meeting that more effective diagnoses carried out in clinics and laboratories in the developing world would, providing accurate statistics were collected, help governments and international agencies towards a clearer view of the importance of intestinal protozoal diseases. This should lead to better targeting of aid and research funds. The development of new molecular biological tools for diagnosis and the implementation of laboratory improvement programmes will play a vital role.

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A full report of the meeting (WHO/CDS/IPI/92.2) is available through the Programme for Intestinal Parasitic Infections, Division of Communicable Diseases, World Health Organization, 1211 Geneve 27, Switzerland, and also from Dr Fernando A Beltrán, Regional Adviser in Parasitology, Communicable Diseases Program, Pan-American Sanitary Bureau, Pan-American Health Organization, 525 Twenty-third Street NW, Washington, DC 20037, USA.

# Audit in the Public Health Laboratory

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## Introduction

I do not know what qualifications it was thought that I had for membership of the Think Tank on PHLS audit, but I do know that the group was asked to tackle a difficult subject at a time when ideas on audit and quality assurance were developing rapidly. Consequently I had to give a lot of thought to the subject during the time that the group met and have continued to think about it since.

'Medical audit' was a buzz phrase in the late 1980s and the flow of articles on the subject has shown no sign of abating in the 1990s. The plethora of audit documents, audit activities and audit committees is such that it is easy to lose sight of the principles and the purpose of the process. The importance rightly attached to it by the Department of Health is reflected in the financial resources being made available for it, and every laboratory should now be forming a view of what audit entails and how it intends to set about meeting its requirements.

## Why must we undertake audit?

A number of internal and external factors will ensure that audit remains a high priority.

1 *'Working for Patients'*[1] signalled the commitment to value for money studies by transferring responsibility for external audit of NHS activity to the Audit Commission.
2 *Purchaser pressure* With the purchaser-provider split, purchasers (health authorities, local authorities, fundholding GPs, etc) will be looking for evidence of cost-effective, high quality services.
3 *Professional* Clinical audit by all health care staff is consistent with professional values such as a commitment to improve the quality of services provided to patients.
4 *Managerial* Many aspects of medical audit are of interest to health managers.[2] The results can determine unit policies for the management of resources and provision of services. It is likely that managers will attempt to increase their involvement in audit.
5 *Commercial* As laboratories increase their contact with commercial organisations, eg by income-generating work, they may find that customers look for information about the quality of service they intend to purchase.

## What must we do?

Audit is here to stay. It is a means to an end and not an end in itself. Audit is part of a larger framework that ensures that a laboratory achieves its objectives. The first step in this process is to define the goals of the department and the objectives of each part of the PHLS network as outlined in the Corporate Plan 1990.[3] Laboratory staff may feel that they need to develop these objectives locally to include reference to areas such as staff development and training, cost effectiveness, safety etc. Laboratories should also define and specify the level of all the services they offer. A system must be implemented that ensures that high standards are built into the work of the laboratory and that all members of staff are aware of what is required of them. In most laboratories much of this is already in place, but it may need to be consolidated. A quality assurance programme covers all aspects of the service offered. It may include policies for the induction and training of new staff, continuing staff development, laboratory user guides, safety policies, procedure manuals, occupational health policies, and equipment and accommodation specifications - as well as the internal quality control and external quality assessment programmes that have been part of laboratory life for many years. Audit is thus the final step in the process whereby we assess whether we are achieving our stated objectives.

The five key questions in the audit process are:

- What should we do?
- What do we do?
- Are we doing what we should be doing?
- Can we improve what we do?
- Have we improved?

This can be represented as a control loop (Figure) which starts by agreeing the standards to be achieved, monitoring what happens and comparing it against the standard. If the standard is not attained, corrective action may be necessary or the standard may need to be reassessed if it is inappropriate.

## Types of audit

Financial audit and the work of the Audit Commission are undertaken mainly by auditors from outside the laboratory, but medical microbiologists have an important role in medical and laboratory audit.

### *Medical audit*

All doctors are required to participate in medical audit, defined as 'the systematic, critical analysis of the quality of medical care, including the procedures used for diagnosis and treatment, the use of resources and the resulting outcome and quality of life for the patient'.[5] Medical microbiologists must be involved in medical audit and in my experience this has taken two forms:

a) Local medical audit as a multidisciplinary activity which may cover topics such as infection control, the appropriate use or otherwise of the laboratory, antibiotic policies and usage. Contributions from microbiologists help to maintain the profile of their department at a time when some may be tempted to economise in this area.
b) Peer review is a fundamental principles of medical audit, even though it is often difficult at district level. The problem has been overcome in some areas by the development of a regionally based audit programme as described by the Association of Medical Microbiologists.[6]

*Laboratory audit*

There have been a number of attempts to analyse the processes that occur in the pathology laboratory, the most recent being the report from the Audit Commission which defined a sequence of objectives which should lead to a high quality service.[7] Laboratory audit is concerned primarily with the everyday aspects of the work of the department and is a means of providing feedback to both the users of the laboratory and its staff. It should be organised internally, although the UK National External Quality Assessment Scheme (UKNEQAS) and accreditation schemes such as Clinical Pathology Accreditation (CPA) and the National Measurement Accreditation Service (NAMAS) can complement the in-house programme of audit.

Areas where the laboratory can take the lead include:

- Request form - is it easy to use? Are all relevant details provided?
- Specimens - is the right specimen received at the right time? Are appropriate investigations selected by laboratory staff?
- Turn-around times - ie the intervals between collection of the specimen, receipt in the laboratory, issue of a report and its receipt by requesting doctor.
- Range of investigations available - is it appropriate?
- Procedure manuals - do they cover all aspects of the work including administration and business activities?
- Use of laboratory out of hours.
- Safety policies and procedures.
- Staff induction procedures.
- Occupational health policies.
- User views on the service provided.
- The efficient use of staff - do senior staff perform duties that should/could be delegated to others?

A number of laboratories have published their experiences of audit.[8-10] The involvement in and commitment of staff to audit is important and there must be a means of co-ordinating the work and presenting it so that the results are shared and provide feedback on performance. There must be a mechanism to correct areas that could be improved and to re-audit to see if improvement has occurred.

## Laboratory audit: the Norwich experience

In Norwich my aim is to develop a quality assurance programme with audit providing feedback on our performance. My interest began at a quality assurance study day where we were asked to describe high quality aspects of the work of our departments. I believed that we offered a good service, but I had little objective evidence of this apart from our UKNEQAS results. My colleagues and I had already sought views from individual consultants who made significant use of the laboratory. We felt that we should also canvas the views of general practitioners as they provide about 40% of our workload. Periodically individual practitioners had criticised particular parts of the service and we felt that it was important to see if this dissatisfaction was widespread. A questionnaire was circulated which sought views on:

i) the general quality of the service
ii) the range of investigations offered
iii) the format of our reports and their timeliness
iv) the ease and usefulness of contact with laboratory staff, including medical, technical and clerical
v) educational opportunities provided by the laboratory.

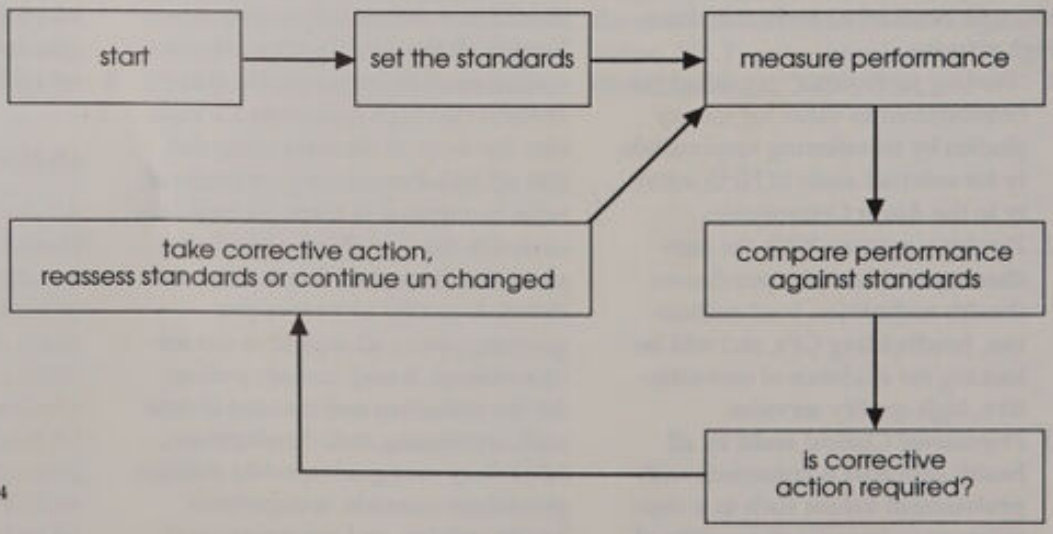


**Figure** Control loop of audit. From Managing Health Services[4]

Replies could be anonymous so that respondents could be highly critical if they wished. It was reassuring that the general level of satisfaction was high. Areas about which we were particularly concerned, such as the speed of our switchboard response and access to staff, were less of a problem than we had thought. The comments have allowed us to address a number of issues - some major, some minor. A single, vociferous complainant can create the impression that the service is far from satisfactory and this can affect staff morale. This survey gave us objective evidence of the views of an important group of our users and, I believe, has improved relationships because we have demonstrated that their opinions are important to us and responded to some of their criticisms.

The PHLS Think Tank developed a programme for audit of food and environmental microbiology and PHLS virology. A volunteer laboratory was needed for a pilot study and Norwich was chosen for this. The group had agreed on standards for the areas to be audited and the exercise began with completion of a questionnaire which looked at staffing levels, relationships with departments of public health medicine and local authorities, computer systems, equipment and the range of investigations available. The other members of the group visited the laboratory to inspect the facilities and to meet staff and users of the service including environmental health officers and community physicians.

In agreeing to the choice of Norwich I was confident that there would be no nasty surprises but there were areas where I knew we could improve. One benefit became very clear during the weeks prior to the visit. With the distinct feeling that they were going on show the staff became very 'house-proud'. The whole laboratory was cleaned and tidied; procedure manuals were updated. Thermometers were bought for all the fridges and incubators and regular recording of temperatures began. The visit went very well. The comments were generally complimentary but, as expected, areas were identified where we could do better either by internal action or with external support. The very positive feedback received from the Think Tank was widely disseminated amongst staff and the increase in morale (including the author's) was tangible. The problem areas have been addressed and some have been resolved while others are being dealt with as part of ongoing projects. All have been approached in a positive way. It is important that one should be prepared to respond positively to constructive criticism.

Staff have agreed appropriate turn-around times for specimens. Our computer manager has extracted the relevant data from the computer and presented us with it in a graphic form, and it is surprising what has been revealed. We will develop a programme to review the turn-around times regularly so that we can show ourselves and our users whether we have achieved our targets.

Our latest development has been to ask the MLSO who collates our UKNEQAS results to co-ordinate quality assurance activities. With the imminent arrival of accreditation he has begun by highlighting areas in the CPA standards where we need to take action. I expect that his role will develop, particularly if we seek accreditation by CPA as well as by NAMAS and, perhaps, BS 5750 at some future date.

## Summary

Medical microbiologists will increasingly be involved in medical and laboratory audit. Local laboratory audit is an internal activity concerned with the aspects of everyday work and must have the involvement and commitment of staff. There must be a means to co-ordinate audit activities and to present the findings to staff. This provides feedback on performance and helps to provide motivation. There must be a mechanism to correct deficiencies and to re-audit to confirm that improvement has occurred and has been maintained.

In the commercial climate in which we now work it is important to identify the needs of the laboratory's users and to listen to their views on the service they receive. We enjoyed the final comment from one of the respondents in our GP survey: 'I must say I am surprised at how satisfied I am' - so were we. A truly satisfied customer.

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# Conference reports

## 5th Congress of the European Society for Organ Transplantation

### Maastricht
### The Netherlands,
### October 1991

CMV infection was the subject of many of the virology papers presented at this meeting. Studies included the prophylactic use of ganciclovir, CMV excretion as a prognostic factor of severe CMV disease, risk factors associated with the development of CMV disease in transplant patients, and the relationship between CMV and the development of obstructive bronchiolitis.

Other papers dealt with the incidence of lymphoproliferative disorders and their relationship to levels of immunosuppression, HIV disease in renal allograft patients, and long-term immunoprophylaxis in hepatitis B positive liver transplant patients.

Eight presentations on hepatitis C virus (HCV) infection attracted much interest. Around half of patients receiving HCV positive organs acquire HCV infection at transplant; 10-15% of these have elevated ALT and most in this group go on to develop liver disease. Organ donors should be screened for HCV antibody.

Finally, the author and colleagues presented a paper describing the effect of introducing pyrimethamine prophylaxis in *Toxoplasma gondii* antibody negative heart recipients of *T.gondii* positive donors. Administration of pyrimethamine resulted in a highly significant reduction in the incidence and severity of *T. gondii* infections in these patients.

***Further information from***
*TG Wreghitt*
*Cambridge Public Health Laboratory*

## EEC Group on Candida Cell Biology Second Annual Meeting

### Valencia, Spain
### 11-14 November, 1991

About 30 representatives from the 11 European laboratories constituting the Group attended the meeting. Through support from the EEC a network has been created which links centres with common research interests in the nature and role of the glycoproteins involved in the cell biology of *Candida albicans*. Exchanges are effected of personnel, methodology, reagents and probes, and co-operative programmes are conducted on the nature and function of cell wall macromolecules, with particular respect to their biosynthesis, immunogenicity and molecular biology.

Among the topics considered were yeast killer toxins, biotyping, antigenic variability, mucosal immunopathogenicity, adhesins, phenotypic switching, serodiagnosis, standardisation of procedures and reagents, and immunomodulatory properties of mannoproteins. Some genes coding for glycoprotein enzymes, notably the aspartic protease of *C.albicans*, have been cloned and sequenced, and a wide range of monoclonal antibodies have been synthesised to different phases and components of the cell wall.

A particularly intriguing recent development in Parma has been the use of antibodies directed against idiotypic antibodies produced against yeast killer toxin. These 'antibiobodies' have the same effect on *Candida* cells as killer toxin itself, but appear to leave mammalian cells unaffected. Their mode of action and potential as a new means of treating candidosis is being investigated. Synthesis of several other cell wall glycoproteins has also been identified as a potential target area for new chemotherapeutic approaches.

Although the meeting began with a review of current research at each of the 11 centres, the principal objectives were to review the co-operative work being done within the network and to plan future studies.

The programme is proving helpful in developing and evaluating new serodiagnostic reagents (including MAbs) and procedures, in testing our own and other systems for recognition of epidemiological markers, and in continuing our joint research on oligosaccharidic epitopes with the Group at INSERM in Villeneuve d'Ascq.

The concept of a network approach to an area of common research interest is not a new one. It has been attempted before in a variety of ways, with varying degrees of success. The likelihood of success is limited unless interests are truly complementary and there is a genuine willingness to work co-operatively.

This Group has now been in existence for some 16 months. It is effective and productive, and the work completed to date has laid the foundation for an expanding range of future research and training activities.

***Further information from***
*DWR Mackenzie*
*Mycology Reference Laboratory*
*PHLS Central Public Health Laboratory*

## International Symposium: Active Immunisation Against Hepatitis A

### Vienna, Austria
### 27-29 January, 1992

Some 800 delegates gathered at the

Intercontinental Hotel in Vienna for this commercially sponsored symposium held to coincide with the licensing of an inactivated hepatitis A vaccine (Smith Kline Beecham Biologicals) in a number of European countries.

Speakers in the early sessions described the clinical features of typical and atypical cases of hepatitis A and the management of the fulminant case with particular reference to transplantation. The properties of the hepatitis A virus (HAV) were reviewed as well as its probable classification in a new genus Heparnavirus within the picornavirus family. Seven genotypes of HAV can be distinguished, three of which are only found in non-human primates.

The history of the HM 175 strain of HAV was described from its origins in Melbourne, together with discussion of the genome changes associated with its adaptation to cell culture. This virus, grown in MRC5 cells and formalin-inactivated, is the basis of the SKB vaccine. Live attenuated vaccines derived from HM 175 have proved disappointing so far; however, this type of vaccine is being used, apparently satisfactorily, in China, employing attenuated H2 strain HAV.

Several speakers reviewed the epidemiology of hepatitis A, the risks of hepatitis A infection in travellers to developing countries, and the problems of hepatitis A in the military. Many vaccine trials were presented. From the available data this vaccine appears to be safe and effective. Given 720 ELISA units of vaccine (1ml) intramuscularly in a three dose schedule, usually 0, 1, 6 months, around 100% seroconversion was reported after the second dose. Similar high seroconversion rates can be achieved with accelerated schedules such as 0, 1, 2 months, or even with two doses a fortnight apart, but geometric mean titres are significantly lower with the abbreviated regimens. Seroconversion rate was unaffected by simultaneous administration of immune serum globulin, although again geometric mean titre was lowered. Titres were not affected by simultaneous administration of hepatitis B vaccine, yellow fever vaccine or typhoid vaccine. Much is not yet known, for example the duration of protection (estimated to be 10 years after full vaccination schedule), the efficacy of the vaccine for post-exposure prophylaxis, immunogenicity in neonates, efficacy by the intradermal route.

There was agreement on a role for the vaccine among travellers, for certain institutionalised individuals, and for those in high risk occupations - military personnel, workers in day care centres and sewage workers. The possibility of mass vaccination of children rather than targeting particular groups was discussed at length. Such an approach might be satisfactory in developed countries. However, the cost and the risk of shifting the age at infection to an older population, thereby increasing clinical illness, makes this idea inappropriate for developing countries.

*Further information from*
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## Visit to Laboratory Centre for Disease Control, Ottawa; Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington; Centers for Disease Control, Atlanta

### 17-25 February 1992

I was invited to contribute to the Laboratory Centre for Disease Control (LCDC, Ottawa, Canada) seminar series, and this provided a valuable opportunity to visit other 'listeriologists' in North America. The work of LCDC involves surveillance of infectious diseases. In addition to completing a surveillance programme for listeriosis, the laboratory was involved with an ongoing outbreak of groupC meningococcal meningitis.
The Health Protection Branch also in Ottawa, has regulatory responsibilities and their current work on *Listeria* includes its survival and growth in food under modified atmospheres or under vacuum, typing *L.monocytogenes* by pulse field electrophoresis, and the experimental infection of primates.

The American Food and Drug Administration is conducting research on the invasion of *Listeria* into a macrophage cell line, ways of improving typing discrimination with polyclonal antisera and nucleic acid-based tests to detect *L.monocytogenes* in food. They are also doing preliminary work on a nucleic acid-based typing system using pulse field electrophoresis. Other current interests include *Clostridum botulinum, Campylobacter* and *Staphylococcus*. Cholera is of growing concern, both in imported carriers and endogenous cases.

At the Centers for Disease Control (CDC) in Atlanta two case control studies on listeriosis had been carried out, together with a survey isolating *Listeria* from food taken from patients' refrigerators and the results of these have now been published *C JAMA* 1992; **267**: 2041-5, 2046-50. Surveillance work on intestinal parasites and outbreaks of waterborne diseases was described. A protocol for performing a new staining technique for microsporidia in stool using chromotrope 2R along with a stained slide have been brought back to Colindale and are available for interested workers to examine.

*Further information from*
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*PHLS Central Public Health Laboratory*

## European Coccidiosis Discussion Group Institute for Animal Health

### Houghton, Huntingdon 20 February 1992

Coccidia are protozoa which include the human pathogens *Toxoplasma, Cryptosporidium, Isospora* and *Sarcocystis*, and a large number of other genera which are important animal pathogens. Of these, the Eimeria have been studied the most. This meeting included work done by researchers from France, Germany, Israel, Hol-

land, the USA and Australia.

Eimeria are a problem in the poultry industry, causing intestinal lesions, reduced weight gain and mortality in young birds. Much of the research has been aimed at reducing these effects through the production of vaccines and by other means. Strategies for vaccinating chickens are constrained by problems of cost and parasite adaption. Attenuated live strains have been added to chicken food at low concentration to induce low level infection and subsequent immunity. Another approach involves the induction of high titre specific antibody production in egg yolk.

Presentations included work on the physical and chemical factors influencing oocyst excystation, the relationship between free radical production and resistance to infection, the immunolocalisation of secretory antigens, and interpretation of ultrastructural features. New approaches to vaccination included the use of specific recombinant antigens, either as polypeptide vaccines or using live *Salmonella gallinarum* or fowlpox virus recombinant vaccines. A study partly funded by the PHLS used a nested PCR to detect presence of *Toxoplasma* in tissue biopsies.

The considerable amount of research on coccidiosis in animals is of growing importance to medical parasitologists with the increase in the immunocompromised population due to HIV infection.

*Further information from*
*GL Nichols*
*Brighton Public Health Laboratory*

## Visit to the Uganda Virus Research Institute (UVRI)

### Entebbe
### February - March 1992

The purpose of this visit was twofold:

1) To assist the Head of the Virology Department and the Consultant Virologist in the training of UVRI technicians in methods for: a) the isolation of HIV from seropositive persons, especially asymptomatic or recently infected people; b) the identification of recently infected persons by detection of HIV antigens and nucleic acids in plasma and lymphocytes and very early serological responses, eg low reactivities on Western blots.

2) To assist in training the technicians in the safe handling of HIV under Category III containment conditions.

The main objectives were to establish a set of routines designed to identify persons who had been recently infected with HIV and to isolate viruses from these and other asymptomatic persons. The following protocol was established with the co-operation of Dr BR Tegume, Head of the UVRI clinic, which is attended by persons for a variety of reasons, many unconnected with AIDS.

Blood samples, about 10 a day, are taken immediately to the laboratory for analysis and people attending the clinic are asked to return two weeks later for the results of their tests, when they may be asked to give a larger (20ml) sample of blood.

The blood samples are tested for antibodies to HIV using a rapid test (Cambridge Biotech) and positive samples centrifuged in leukoprep tubes (Becton Dickinson) to obtain lymphocyte and plasma fractions. Plasma from all the blood samples are stored at -20° and lymphocytes from seropositive samples are stimulated with phytohaemagglutinin and cultured for 4 weeks with twice-weekly feeds. Cultures are tested twice weekly for the production of p24 antigen using a commercial kit (Coulter). Cultures positive for p24 antigen are passed onto uninfected cord blood lymphocytes and the cultures examined daily for the production of syncytia and weekly for the production of p24 antigen. Supernatants from the primary and cord lymphocyte cultures are aliquoted and stored at 80°: cells from these cultures are stored in foetal calf serum plus 10% DMSO in liquid nitrogen.

The plasma is tested for HIV-specific antibodies by two enzyme immunoassays - the Wellcozyme HIV Recombinant assay (Wellcome) and the Recombigen HIV EIA assay (Cambridge Biotech). Plasma giving discordant results - ie, only one positive reaction - are examined by Western blot (Cambridge Biotech) and those giving very weak or restricted profiles - especially a weak reaction with gp160 or gp120 - are re-examined by Western blot followed by an enhanced detection system, currently either the ECL kit from Amersham or the Dupont Blast system. Sera are also assayed for p24 antigen, after dissociation of immune complexes. Weak or restricted Western blot profiles, with or without p24 antigen, indicate a recent infection and the technician in the clinic is asked to obtain a second, larger blood sample.

Approximately 180 blood samples were obtained from the clinic during the course of the visit: about 25% of these were seropositive.

Forty samples of lymphocytes from seropositive persons were cultured: most grew extremely well but only two virus isolates were made, possibly indicating a low virus load in most of these asymptomatic persons. Culture supernatants from the two isolates were aliquoted and stored at 80° and samples of each were passed onto cord blood lymphyocytes. Both produced syncytia within 2 weeks and aliquot of the cells and supernatants were stored in liquid nitrogen.

Four technicians helped in the virus culture work: they worked well and one is extremely proficient and experienced. Two technicians did the p24 antigen assays, producing reliable data.

One technician was trained to use the amplified Western blot systems but the results were not consistent and more time will be required before either method is established as a routine. However, it was felt that an amplified Western blot method would be very useful in identifying recent sero-converters.

One of the objectives was to train a technician in the isolation of RNAs from plasma and increase his experience in PCR methods. This

objective was not fully realised but some progress was made and the UVRI staff are now very keen to get PCR established for both RNA and DNA templates.

The category 3 Virus Containment Laboratory was still being built so it was not possible to train technicians in class 3 safety cabinet work.

A distilled water lysis method for the rapid isolation of DNA from whole blood was modified to give a cleaner product in better yield. This should find use in the MRC Field Station and in other up-country laboratories.

A number of samples of RNA, DNA and plasma were brought back to the UK and will be used in nucleotide sequence and serological studies.

The power supply was unreliable, with frequent cuts and low voltages during the first two weeks of the visit. This caused considerable problems in the PCR work, which were only partially solved by use of the Institute generator. As the power supply problem also affects many other aspects of work in the HIV laboratory - the operation of the safety cabinets, centrifuges and the ELISA reader - a smaller modern generator is urgently needed for the HIV work. In the event of a power failure, the present system is unsafe. Some consumable items were in short supply but UVRI staff are expert in coping with such problems.

Overall, this visit was very successful. The new routine was established and should improve in efficiency if the incentive system is maintained. The UVRI clinic will probably sample about 2 500 people a year. Assuming a seropositivity of about 25% and a yearly incidence rate of about 2.5%, about 100 of these people will have been infected within the past year and about 8 within the past month - ie those of greatest interest in a molecular epidemiology study. It should be possible to identify some of these people and obtain samples and isolate viruses from them.

The kind help and encouragement of the Director of UVRI, Dr S Sempala and the Head of the Virology Department, Dr B Biryahawaho and their staff was gratefully acknowledged. The visit was funded by the AIDS Directed Programme of the Medical Research Council.

***Further information from***
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*PHLS Centre for Applied Microbiology and Research*

## Fifth International Conference on Human Retrovirology: HTLV

### Kumamoto, Japan
### May 1992

This conference was attended by about 300 delegates, most of whom were from Japan, North America and the West Indies. This was about twice the number that attended last year, reflecting growing interest in the field. The conference was held in Kyushu Island, Japan where in certain areas 15% of the population are infected with HTLV and the annual incidence of adult T cell leukaemia/lymphoma (ATLL) is >5/100,000.

***Molecular epidemiology***

Several groups of workers had investigated the molecular epidemiology of HTLV, using either sequence analysis or restriction enzyme patterns. Unlike HIV, the sequence divergence of HTLV-I was shown to be highly restricted and closely correlated with geographical source. Nucleotide changes at the same position were shown to be diagnostic for the geographical origin of the virus. The least variability was found in the *env* region, and the most in the LTR region. No specific variations were associated with ATLL or tropical spastic paraparesis (TSP).

Isolates from West Africa, the Caribbean and South America, and from Japan, showed very little sequence diversity and these were referred to as 'classical strain' or prototype. Isolates from Iran differed in their nucleotide sequences from this strain by about 1%, and isolates from Zaire differed by 3%. A Zairian-like isolate has also been made in Sicily. Novel and highly divergent isolates from Melanesia, showing 8-9% sequence divergence from the prototype strain, were described by several workers. A variant isolated from Papua New Guinea differed by 4% from a variant from the Solomon Islands. Other variants have been isolated from Australian aborigines. By comparing sequences of the different strain variants and constructing dendrograms in which the percentage difference between strains represents a time scale, Yanagihara and colleagues concluded that the Melanesian variants of HTLV had diverged from primate T cell lymphotropic virus prior to the cosmopolitan HTLV-I strain and African sybtypes of Simian T cell lymphotropic virus type-I (STLV-I). This implies that Africa could not have been the origin of the virus. Koralnik et al compared STLVs isolated from primates from Africa and Asia and reported a close relationship between African STLV-I and HTLV-I, the closest being a chimpanzee virus isolate from Sierra Leone and a virus isolated in Uganda from *Cercopitecus ascanius*. These showed 3.1% and 3.4% divergence from HTLV-I, respectively. Asian monkeys harboured STLV-I virus which showed up to 15% divergence from HTLV-I. Several of the eight papers on this subject included a map of the world showing interpretations of how the spread of early man across the continents could be linked with the dissemination of HTLV. It is difficult to draw a definite conclusion on the origin of HTLV from the evidence available to date. It seems likely that interspecies transmission occurred, and clusters of strain variants, for example in Zaire, may have arisen from infection with STLV-I. Studies on the strain variants will be important in vaccine development and in the search for new endemic areas of HTLV-I/II.

***Serodiagnosis***

Virtually all presentations emphasised the difficulties that have been encountered in confirming the reactions of sera in HTLV antibody screening assays. Until recently, the

lack of sensitivity of Western blot in detecting antibodies to *env* products has necessitated use of RIPA, a very laborious test. Although the addition of recombinant p21e antigen to conventional blots has increased their sensitivity, false positive reactions against p21e were reported by several groups including our own. Results using blots which incorporate specific HTLV-I and HTLV-II recombinant proteins were encouraging, and I understand that these will be commercially available within the next two months.

Some seroepidemiological studies ignored accepted criteria for interpretation of Western blot, and other groups suggested less stringent criteria which they claim will increase the sensitivity of assays. They gave little information on the rate of false positive results using such criteria.

*Transmission*

The focus was on vertical transmission of HTLV-I, and almost all presentations were of Japanese studies. As has been known for some time, the major route of transmission is from mother to child via breast milk. So-called ATL prevention programmes, in which HTLV antibody positive pregnant women are advised against breast-feeding, are in progress in Japan. The effect of these interventions has been considerable. In Nagasaki, for example, the rate of maternal transmission of HTLV-I has been reduced from about 20% to about 3%. Other groups reported similar proportions of children of infected mothers being infected without exposure to breast milk (4/86 (4.7%) Saito et al, 7/540 (3.1%) Kawase et al). Fujino and colleagues suggested that this group may have been infected through intrauterine transmission since *in vitro* experiments have shown the HTLV-I can infect placental villous cells. This suggests the possibility of prenatal transmission via trophoblasts at the materno-fetal interface. Studies using PCR, however, suggest that infection does not take place *in utero* as the presence of HTLV-I DNA in cord blood does not predict transmission. Hino and workers reported that none of five children of carrier mothers in whom HTLV-I genome was detected by PCR in cord blood was subsequently shown to be infected and none of four carrier children had had PCR positive cord blood. Likewise, Kawase et al reported that none of eight children with PCR positive cord bloods showed evidence of infection at age 12 months. It therefore seems more likely that transmission of HTLV-I, other than by breast-feeding, occurs at birth. This is consistent with the now widely held view that most maternofetal transmission of that other important human retrovirus, HIV, occurs at delivery.

Two reports suggested that passively transferred maternal antibody may have some protective effect against HTLV-I infection of the infant. Kawakami et al reported that the seroconversion rate in children of carrier mothers who were breast-fed for more than 7/12 was 11.8% (19/161), but was only 3.5% (4/113) in children who were breast-fed for less than 6/12. In a study of children born to carrier mothers in Jamaica, Wiktor et al reported that 27/220 seroconverted. Maternal antibody persisted for 7.3 months (mean) 7.0 - 7.4 (range) and seroconversion occurred at 14.1 months (mean) 9.8 - 21.5 (range).

Nakachi et al reported that the presence of antibody to p40 tax in carrier mothers may be related to their infectivity. 3/10 breast-fed children born to p40 antibody positive mothers were found to be infected with HTLV-I, whereas 0/14 breast-fed children of p40 antibody negative mothers were found to be infected. On the basis of these results, Nakachi suggested that mothers lacking antibody to p40 were regarded as low risk. He recommended that they should breast-feed their infants. This seems a small number of results from which to draw this conclusion. Shiori et al reported that the seropositivity rate of wives of p40 antibody positive men (81.1% (60/74) was significantly higher than that among wives of p40 negative men (65.3% (32/49), $p = 0.049$).

In the opening lecture, Miyoshi presented the results of passive immunisation of rabbits born to HTLV-I infected dams using human and rabbit HTLV-I immunoglobulin. One of 12 HuHTLV-I Ig inoculated rabbits, compared with 6 of 14 control rabbits, and none of 15 Rabbit HTLV-I Ig inoculated, compared with 9 of 16 control rabbits, seroconverted. These results suggest that passive immunisation is effective in preventing dam to offspring transmission of HTLV-I.

Little data was presented on transmission of HTLV-II. However, Kaplan et al reported that 20 non-breast-fed babies of HTLV-II antibody positive mothers showed no evidence of HTLV-II infection by PCR at 0 - 36 months.

***Cofactors for the development of TSP and ATLL***

Only a small proportion of carriers of HTLV-I develop TSP or ATLL. Several studies attempted to identify significant cofactors. Statistical analysis showed the following cofactors to be associated with either TSP or ATLL: age of first intercourse less than 18 years (Kramer et al), single marital status (Maloney et al), subject drank more than one cup of bush tea/week (Kramer et al), low level of education (Maloney et al), smoking (Tokudome), frequency of blood transfusion (Tokudome), intake of green/yellow vegetables and of soyabean curd linked with lower incidence of disease (Tokudome).

In a study of HTLV and TSP in Zaire, Goubau et al reported that although the virus was evenly distributed between ethnic groups, TSP clustered both in families and in ethnic groups. The Mondunga tribe had a high rate of TSP, but no obvious characteristics of the tribe could be identified that might account for this.

***Clinical studies***

Much attention was focused on the clinical effects of TSP and ATLL, and on the search for other diseases associated with carriage of HTLV. One 10-hour session was devoted to the definition of ATLL. The manifestations of HTLV-I included: proliferative synovitis, uveitis, mental illness, Hashimoto's throiditis and infective dermatitis.

*Non-endemic areas*

Studies from non-endemic areas such as Europe were few in number and focused largely on the decision to screen blood donors for HTLV. Screening was instigated in Australia three weeks prior to the conference, probably in view of the isolation of a strain variant from Aborigines. It was reported that as many as 10% of Australian Aborigines may be infected, but further studies are being hampered by complex ethical issues. As far as is known, France is the only European country to screen all blood donors for anti HTLV-I. However, HTLV-I and HTLV-II infections associated with blood transfusion were reported from Italy.

*HTLV-II*

No specific diseases have been associated with carriage of HTLV-II. A study of 73 HTLV-I and 166 HTLV-II carriers showed that about 10% of carriers of both viruses had abnormalities in gait and vibration sense, that poor health status was seen more frequently in HTLV-II carriers than in controls and that 'flower cells', ie multilobulated white cells, were seen on blood smears from 31% of HTLV-I and 23% of HTLV-II carriers. In a separate study, Murphy et al showed that HTLV-II may be related to neurologic disease and arthritis, and may be a cofactor for pneumonia, anaemia and lymphadenopathy among IVDUs.

HTLV-II was reported by several groups to be endemic in isolated American Indian tribes, as well as in IVDUs throughout the western hemisphere. In North America epidemiological studies of STD patients and IVDs conducted since 1988/9 have not shown an increase in seropositivity. The distribution of HTLV and HIV infection in these populations was significantly different. HTLV rates in IVDUs were highest in the West, ranging from 0.4% in Atlanta to 17.6% in Los Angeles. In contrast, HIV rates were highest in the East (28.6% in Newark). Sequence analysis by Takahashi et al showed that there were two distinct subtypes of HTLV-II, showing 4-6% divergence. The greatest divergence occurs in the LTR region.

This meeting brought together important data on all aspects of the biology of HTLV-I. This virus is of public health importance in England and Wales where a small but significant focus of HTLV-I and HTLV-II infection exists.

***Further information from***
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# COLINDALIA

**Grave matters**

Last month an undertaker involved in the exhumation of a body went to his general practitioner to ask whether his men should be vaccinated against smallpox 'just in case'. He was obviously an attentive reader of the *Communicable Diseases and Environmental Health in Scotland Weekly Report* and had noticed the detailed account by J King (vol 26 92/06) of the construction of a relief road at Kirkintilloch running through the disused (since 1927) Orchard Burial Ground. Mr King is the local environmental health officer. Records suggested that in the mid 19th century some smallpox victims had been buried there and though, on that occasion, no construction personnel were vaccinated wide consultation and careful evaluation preceded the work. After much discussion and correspondence between all the agencies involved, a code of practice covering general grave digging, manual grave digging, general conditions, shuttering and exhumations was eventually agreed. The work, authorised in January 1984, was eventually completed within seven months in May 1988. Twenty-nine intact coffins were found. All the remains were reinterred in another cemetery, after being sprayed with White Farm Disinfectant (final concentration equivalent to 1% phenol).

Has smallpox ever been transmitted from a disturbed burial? There seems to be no record of it. Infections in mortuary staff from fresh corpses are well documented, but the principal authorities (Dixon 1962, Fenner 1970) are silent on a risk from longstanding burials. After a burial interval measured in years the risk of primary vaccination of diggers would seem to be greater than the extremely remote smallpox risk, and this was presumably the basis of the decision not to vaccinate taken at Kirkintilloch. In the absence of data it is rather more difficult to assess the need for other precautions such as the respirators used there.

The management of disturbed burials continues to be a perplexing problem for all concerned.

**A Put-down**

Colindale may be the centre of the known world, at least for some of our readers; but definitely not for other people. Novelist Frederic Raphael recently told the following story on a radio show when asked to describe a 'put-down'. Seeking to travel with heavy luggage from Central London to his sister-in-law's he asked a taxi driver to take him to her home in Colindale. `Colindale!' exclaimed the taxi driver, 'I don't go that far for my holidays!'

**An important omission**

A paper by PHLS colleagues and others in Leicester (*British Medical Journal*, 21 March 1992) reports a cluster of 17 cases of hepatitis B after open heart surgery assisted at by an HBeAg positive surgical trainee. They quote a paper by Beridge and co-authors in the *British Journal of Surgery* (1990, 77, pp585-86) which found that of 206 vascular surgeons in Britain, 37% had not had hepatitis B vaccine: 45% of those who had been immunised had not then had their immunity checked. Our colleagues comment mildly `This omission is of importance not only for surgeons' own health but for that of their families and their patients'. Just so!

**Auto-obituary**

A recent innovation by the *British Medical Journal* is to allow doctors to submit their own obituaries, to be published at their death. This is a double-edged freedom which sometimes leads to a banal and colourless account. Occasionally, however, a poet emerges. The best example to date, in which a self obituary surprised and delighted, took the form of a tribute in verse to an antecedent who lived in Glencoul, Sutherland. Readers are referred to the edition of 29 February 1992 for an obituary as out of the ordinary as the leap day. The poem describes the gift from the philanthropist Andrew Carnegie of £5, the purchase with it of 100 Everyman Library classics for the village school, the fate of the schoolmaster and his pupils, and the special pleasure derived from reading 'Vanity Fair'. There the author found his own obituary, namely that 'He was a man of charming gravy-tea'.

**Boiling over**

It has previously been suggested in Colindalia that bringing suspect tap water to the boil in an electric kettle with an automatic switch is a satisfactory and highly convenient way of making it safe. Dr Dadswell draws our attention to a letter in the *Lancet* of 17 August 1991 by Rice and Johnson describing experiments on river, lake and tap water intentionally contaminated with *V. cholerae* and with *E. coli*. Under the most turbid conditions they found that 30 seconds at a rolling boil inactivated both organisms. The `thermal death point' (temperature required to totally inactivate in 10 minutes) was 62°C. Rice and Johnson suggested that one minute of rolling boil would inactivate all pathogens, a more conservative recommendation than the use of the automatic kettle. The same recommendation ('vigorous boiling for one minute') is to be found in Department of the Environment Circular 24/91 as the temporary solution for treating an unsatisfactory supply (`Private Water Supplies WO 68/91'). As has been said before, high altitude alters the case (water boils at 90° C at 3000 metres), though this will not be a significant consideration in the UK.

This controversy has now come full circle, for the question originally posed was whether it is more safe to settle for a brief 'bringing to the boil' or to have water boiling in vessels in a domestic environment. Your diarist remains unconvinced that, all hazards considered, sustained boiling is the preferable option. (Topic now closed.)

**The good news**

A recent article (Klein, *New England Journal of Medicine*, 12 March 1992) draws attention to the extraordinary effect on diphtheria morbidity and mortality in pre-War New York of universal pre-school toxoid immunisation. This immunisation was done 'regardless of ability to pay'. By contrast, the British were at that time being dilatory in making diphtheria toxoid freely available. In 1940 our Minister of Health told the House of Commons that the average annual number of cases of diphtheria notified in England and Wales was about 60 000, 54 000 of them being in children. On average 2 700 children died annually (*Lancet*, 1940, volume 2, page 702). In a report to the Medical Research Council Russell wrote that 'the present rates ... in Great Britain may be held to represent a needless and extravagant waste of child life'. Soon after this diphtheria toxoid did become universally available in Britain and diphtheria quickly became a rarity.

As a result of hard won arguments over the years, diphtheria, tetanus, pertussis, poliomyelitis, measles, mumps and rubella immunisations are now all available to infants in UK. Of these diseases only pertussis remains a substantial public health problem, as a result of imperfect uptake, not of poor vaccine provision.

**Facts and Truth**

For the scientist research is the accumulation of new knowledge: for the historian it is about revealing and interpreting old knowledge and ferreting out the facts that were hidden or distorted by contemporaries. The same care in the conduct of research is required of both, and each can learn from the other. It is because Claire Tomalin's book `The Invisible Woman' (about Charles Dickens' mistress) is so meticulously researched and her interpretation of her data so sympathetic and plausible that it can be warmly commended to scientists. It is conveniently available as a paperback (Penguin Books) for your summer holiday reading.

Dickens was 45 years old in 1857 when he met and became infatuated with a young woman of 18 years, Ellen (Nelly) Ternan. Unfortunately for him his position as the foremost literary champion of family life made it difficult to do what most Victorian men of substance did when they found themselves similarly placed, namely set the object of their passion up in a suburban villa where she could be visited at will. In any case, the object herself was one of three intelligent daughters of a professional but respectable actress and would not easily have fallen in with such an arrangement. Moreover, Dickens `couldn't even get married on account of he had a wife already'. He also had numerous children and a wide circle of family friends.

The situation was one in which Dickens' own scruples and those of the girl and her family admitted no easy solution. He was not, however, a man to be denied and Nelly was susceptible for financial as well as emotional reasons. What the two main players' backgrounds were, how the affair developed, how it ended at Dickens' death in 1870, how it was covered up by Nelly, her family and his, and how it eventually came to light are the engrossing components of this book

A biography of a woman who but for Dickens would be of no historical interest needs a justification. It is that the circumstances of her life, so well told, speak for the period between the mid 19th and the mid 20th century when `respectability' was the overriding British virtue. Nelly's mother, though she sprang from the disreputable world of the Regency Theatre, aspired to and almost achieved respectability. Dickens promoted it in his outward life, but could not tolerate its constraints. Nelly, at his death, managed to conceal a decade of her life and so find respectability in marriage to a young clergyman. Sadly the clergyman then let her down by failing to provide her with the financial security that respectability requires. Her son regained respectability as an officer in an infantry regiment, but was tormented by the revelation, at his mother's death, that she had hidden from him, his sister and his father a long liaison with Dickens.

A scientific fact has previously been defined in these columns as an observation that can be repeated in another laboratory. Historical facts, as shown by Dickens' secret life with Nelly Ternan, are constantly subject to revision. The following anecdote reflects further on this important distinction between facts and the truth.

`I said to him, with all deference, I thought there were greater things in the world than facts. He turned on me and said, `But facts are truth, and truth is Facts.' I said, `No, pardon me; if I may say so, truth I take to be the broad heaven above the petty doings of mankind which we call Facts.' He gave me a smile of pity for my youth, as I suppose, and then said, `Ah well, if ye like to talk in that poetic way, ye may; but ye'll find it in your best interest, young man, to stick to Fahcts.'

(Can anyone, by the way, tell Colindalia who `I' and `him' were?)

# Notes for contributors

## Editorial Panel

Articles sent to the Microbiology Digest are reviewed by the Editorial Panel and will be edited prior to publication. If alterations, other than very minor ones, are indicated, authors will be consulted before the article is published.

## Typescripts

Articles should be approximately 1,500 - 2,000 words long. It is helpful if manuscripts are typed on one side of A4 paper, double spaced and with wide margins. Manuscripts may, in addition, be provided in WordPerfect 5.1 on 3½" floppy disk.

## Illustrations

Illustrations are welcome. Photographs should be clear, glossy black and white prints. Figures, charts and rough diagrams can be redrawn.

When typescripts are prepared, each drawing, table or graph should be on a separate page, with these pages grouped together at the end of the main article. Captions should be supplied for all illustrations, which should be referred to in the text. Figures and photographs should show on the back the author's name and the figure number. Where necessary, the top of the figure should be marked 'top'.

If illustrative material has been borrowed from another source, permission to reproduce it must be obtained and the source acknowledged.

## References

Authors should use the Vancouver style for references, as outlined in 'Uniform requirements for manuscripts submitted to biomedical journals,' *BMJ* 1991; **302:** 338-41. This now requires that the names of the first **six** authors are listed, followed by 'et al'.

## Proofs

Authors will be sent proofs for essential corrections. They are asked to check the text carefully, paying particular attention to references, quotations, tables and figures, and return the corrected proofs as quickly as possible.

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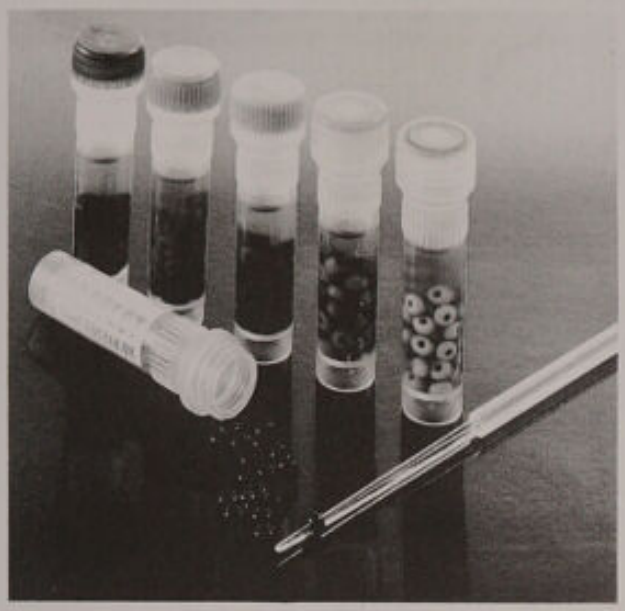

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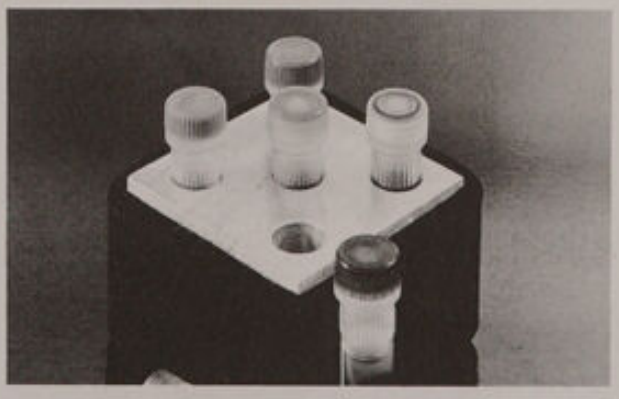

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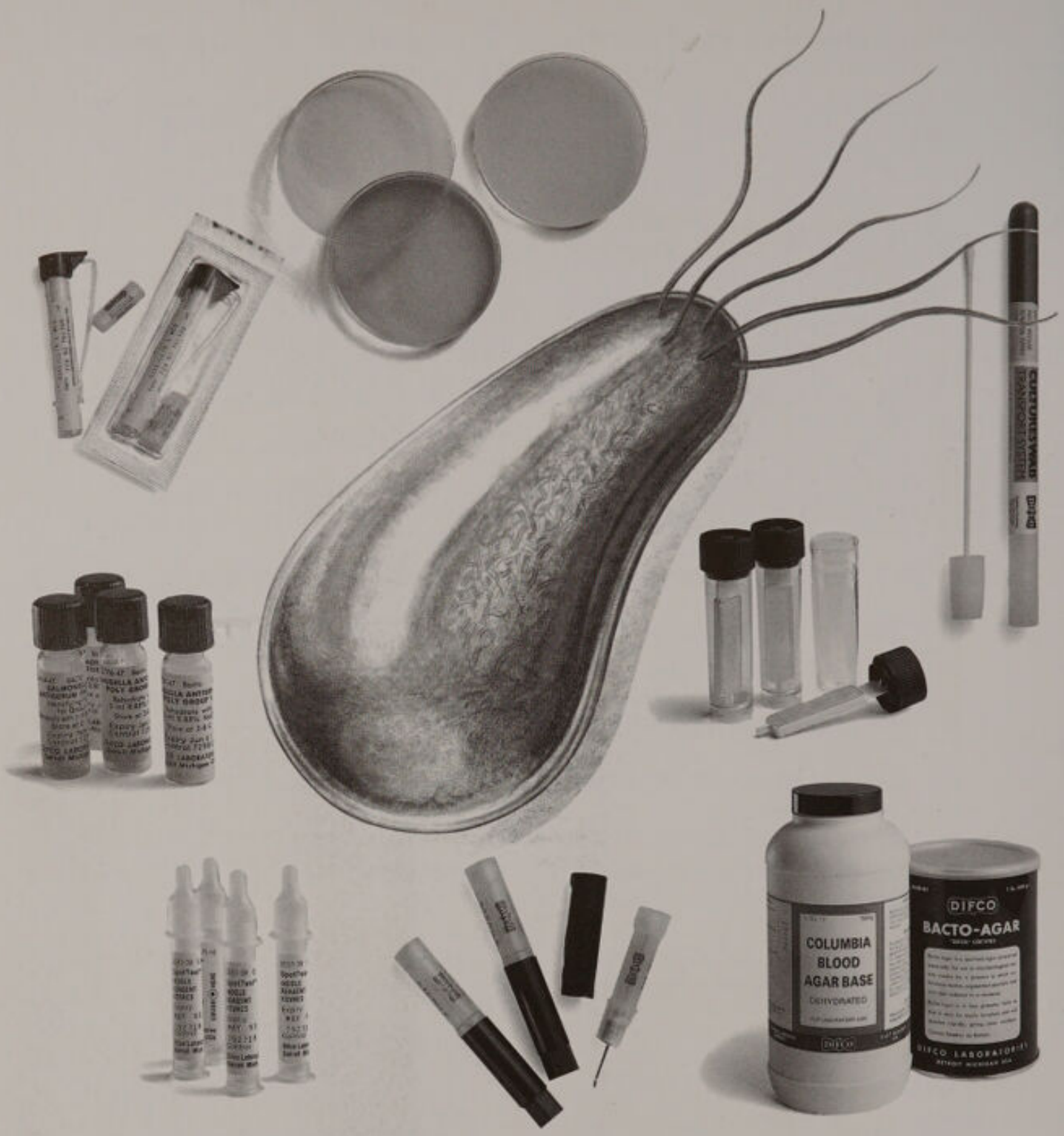


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Communicable Disease Report

# Exposure to hepatitis B virus: guidance on post-exposure prophylaxis

*PHLS Hepatitis Subcommittee*

## Summary

This paper summarises the views of the PHLS Hepatitis Subcommittee on prophylaxis after exposure to known and potential sources of hepatitis B virus (HBV) at work and in the community, and expands on the guidance on hepatitis B immunisation and the prevention of occupational exposure to blood given elsewhere[1,2]. It defines significant exposure and gives guidance on incident recording, risk assessment, testing and storage of incident-related blood specimens and follow-up. It recommends that HBV prophylaxis should be determined by assessment of the likely infectivity of the source and of the HBV status of the person exposed.

## Background and definitions

The risk of HBV transmission associated with an exposure incident depends on the type of exposure (significant or otherwise), the HBV status of the source (infective or not), and the HBV status of the person exposed. Assessment of these will determine the need for, and choice of, post-exposure prophylaxis.

### Significant exposure

A significant exposure is one from which HBV transmission may result. It may be:

(i) percutaneous exposure (needlestick or other contaminated sharp object injury, a bite which causes bleeding or other visible skin puncture)
(ii) mucocutaneous exposure to blood (contamination of non-intact skin, conjunctiva or mucous membrane)
(iii) sexual exposure (unprotected sexual intercourse).

Percutaneous exposure is of higher risk than mucocutaneous exposure, and exposure to blood is more serious than exposure to other body fluids. HBV does not cross intact skin. Exposure to vomit, faeces, and sterile or uncontaminated sharp objects poses no risk. Seroconversion after a spitting or urine spraying incident has not been reported.

### HBV status of the source

This may be:

(i) *known*: an identifiable individual for whom a positive HBsAg test result has been documented in the preceding 12 months or
(ii) *determinable*: an identifiable individual whose HBV status is unknown, but who is available for HBsAg testing or
(iii) *unknown and not determinable*: source unknown, unidentifiable, or identifiable but not available for HBsAg testing (eg, community needlestick injuries, needles from sharps disposal box, community assaults).

### HBV status of the exposed person

The exposed person will be categorisable as:

(i) a known responder to hepatitis B vaccine (post-vaccination level of anti-HBs ≥10miU/ml) or



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(ii) a known non-responder to hepatitis B vaccine (anti-HBs at 2-4 months post-vaccination <10miU/ml) or
(iii) of unknown HBV status: an individual with no history of hepatitis B immunisation, or an individual who has received a complete or partial course of hepatitis B vaccine but without a documented post-vaccination anti-HBs test result, or
(iv) known to be HBsAg positive (this will be rare).

## Incident reporting in the workplace

Employers have a duty to educate staff about occupational hazards and staff should be made aware of local procedures for reporting exposure incidents. Early reporting of incidents is desirable. Hepatitis B vaccine has been available for ten years, and an increasingly high proportion of those health service staff at greatest risk of occupational exposure to HBV will have received a full course of hepatitis B vaccine and will know that they have responded immunologically. These staff must also be encouraged to report exposure incidents, as a booster dose of hepatitis B vaccine may be indicated and as the incident may present a risk of transmission of blood borne viruses other than HBV.

## Incident recording and risk assessment (Tables 1 and 2)

When an exposure incident is reported a history should be obtained. This should be sufficiently detailed to allow an assessment of risk to be made. It should be documented, as this will facilitate follow-up and provide a record for administrative and accident prevention purposes. Points which should be covered are shown in table 1.

The aim should be to complete risk assessment and administration of HBV prophylaxis (when indicated) within 24 hours of the initial report of the incident. It will not always be possible to achieve this but, in any event, the process should be completed within 48 hours. Occasionally, the delay between the exposure incident and the initial report may exceed seven days. There is no good evidence that administration of hepatitis B immunoglobulin (HBIG) would then be beneficial but use of hepatitis B vaccine may still be appropriate.

## Serological testing and storage of serum

### (i) From the source

If the source is identifiable and available for testing, a blood specimen should be obtained (regardless of any previously documented serological test results) and a serum sample stored for a minimum period of two years. This is because it may be helpful to have access to stored serum should the person exposed subsequently be found to have serological evidence of HBV infection, and because the potential for transmission of other blood borne viruses may remain even when the source is known to be HBsAg negative.

Specimens from individuals of unknown HBV status should be tested promptly for HBsAg. Most laboratories will be able, if told of the urgency of the request, to provide the result on the same day. Decisions about the need for HBV prophylaxis can then be based on the result of the HBsAg test and prophylaxis, if necessary, given within 24 hours of the incident report. Where a test result will not be available within 24 hours of the incident report, the incident should be managed initially as for exposure to a known HBsAg positive source. Where the source is an individual known to be HBsAg positive at the time of the incident, immediate HBsAg testing of the sample will not be necessary. In the unusual situation where the source is an individual

**Table 1 Accidental exposure to HBV: points to cover in the incident record***

- **Person exposed:**
  - name, date of birth, sex
  - occupation
  - contact telephone number and address
  - name, address and telephone number of GP
  - hepatitis B vaccine history
  - dates and results of tests for anti-HBs
- **Exposure:**
  - date and time of incident
  - date and time of report
  - place (eg, hospital/community)
  - exposure (percutaneous/sexual/mucocutaneous/other)
  - material involved (blood/other body fluid)
  - further details (including risk of other blood borne viruses)
- **Source:**
  - identifiable/unknown
  - for identifiable sources:
    - name, date of birth, sex
    - contact telephone number and address
    - hospital number or
    - name, address and telephone number of GP
    - results of tests for HBsAg and HBeAg/anti-HBe

* Copies of a sample form are available from the Hepatitis Section, CDSC

**Table 2 Management of reported HBV exposure incidents**

1. Record details of exposure incident (Table 1)
2. Assess significance of exposure:
   - if significant, obtain blood specimen (>5ml serum) from person exposed and arrange testing and/or storage
   - if non-significant, assess likelihood of future exposure to risk
3. Assess infectivity of source
   - if source identifiable and available for testing, obtain blood specimen for urgent HBsAg test and/or storage
   - if source refuses consent, manage as though exposure to HBsAg positive source
   - if source unidentifiable or unavailable, manage as exposure to source of unknown HBV status
4. Assess HBV susceptibility of person exposed
5. Give initial HBV prophylaxis (Tables 3 and 4) (HBIG is not indicated more than 7 days after the incident)
6. Arrange follow-up appointments for further doses of hepatitis B vaccine
7. Arrange follow-up appointment at 6 months post-exposure to obtain final blood specimen from those with significant exposure or who are given hepatitis B vaccine for continuing risk

**Table 3 HBV prophylaxis for reported exposure incidents**

| HBV status of person exposed | Significant exposure: HBsAg positive source | Significant exposure: Unknown source | Significant exposure: HBsAg negative source | Non-significant exposure: Continued risk | Non-significant exposure: No further risk |
|---|---|---|---|---|---|
| ≤1 dose HB vaccine pre-exposure | Accelerated course of HB vaccine* HBIG x 1 | Accelerated course of HB vaccine* | Initiate course of HB vaccine | Initiate course of HB vaccine | No HBV prophylaxis Reassure |
| ≥2 doses HB vaccine pre-exposure (anti-HBs not known) | One dose of HB vaccine followed by second dose one month later | One dose of HB vaccine | Finish course of HB vaccine | Finish course of HB vaccine | No HBV prophylaxis Reassure |
| Known responder to HB vaccine (anti-HBs ≥10 miU/ml) | Booster dose of HB vaccine | Consider booster dose of HB vaccine | Consider booster dose of HB vaccine | Consider booster dose of HB vaccine | No HBV prophylaxis Reassure |
| Known non-responder to HB vaccine (anti-HBs <10 miU/ml 2-4 months post-vaccination) | HBIG x 1 Consider booster dose of HB vaccine | HBIG x 1 Consider booster dose of HB vaccine | No HBIG Consider booster dose of HB vaccine | No HBIG Consider booster dose of HB vaccine | No HBV prophylaxis Reassure |

* An accelerated course of vaccine consists of doses spaced at 0, 1 and 2 months. A booster dose is given at 12 months to those at continuing risk of exposure to HBV.

who is unwilling to provide a blood specimen, the exposure incident should be managed as for exposure to a known HBsAg positive source. This is because those who refuse to provide a specimen may be more likely to be HBsAg positive than those who consent to testing.

### (ii) From the person exposed

An initial post-exposure blood specimen should also be obtained from the person exposed (regardless of their HBV status) and serum stored for a minimum period of two years. It has been the practice in some laboratories to test this specimen immediately for HBsAg. The logic is, that if the exposed person is HBsAg positive, HBV prophylaxis will not be required. In fact, the chance of the person exposed being found to be HBsAg positive will usually be very small. It was recently shown that, of nearly 3000 UK health service staff tested in one centre for HBsAg after reporting a significant exposure incident, only one was HBsAg positive[3]. Testing of exposed persons for HBsAg is, therefore, not cost effective.

If the person exposed has never received hepatitis B vaccine, or has previously received only a single dose, measurement of anti-HBs levels in the initial post-exposure specimen is also unlikely to be useful. Measurement of anti-HBs levels may be worthwhile in those exposed persons who have previously received two or more doses of vaccine. It will not always be possible, however, for laboratories to provide a same day anti-HBs result, and for this reason initiation of HBV prophylaxis for a reported exposure should not be delayed for more than 24 hours from the initial incident report. The cost of providing an urgent anti-HBs testing service – which may have to operate out of hours – should be balanced against any likely savings in HBV prophylaxis. Where the person exposed can be shown to have had a level of anti-HBs ≥100miU/ml† at or around the time of the exposure, further HBV prophylaxis for the incident will not be required.

## Post-exposure prophylaxis (Tables 3 and 4)

### (i) After significant exposure to an HBsAg positive source

Those exposed persons with no history of having received hepatitis B vaccine, and those who have previously received only one dose of vaccine, should be offered a single dose of HBIG (Table 4) and an accelerated course of hepatitis B vaccine. The latter consists of vaccine doses at 0, 1 and 2 months; a booster dose is given at 12 months to those at continuing risk of exposure to HBV. HBIG and the initial dose of hepatitis B vaccine should be given concurrently at different sites. Hepatitis B vaccine should normally be given intramuscularly into the deltoid region in adults and the anterolateral aspect of the thigh in infants.

Exposed persons who have previously received two or more doses of hepatitis B vaccine, but who are of unknown HBV status, should be offered a dose of vaccine at the time of the incident, followed by a further dose one month later. Known responders to hepatitis B vaccine should be offered a single booster dose of vaccine. Known non-responders to hepatitis B vaccine should be offered a single dose of HBIG within 24 hours of the report of their exposure. A booster dose of hepatitis B vaccine may be offered at the same time.

### (ii) After significant exposure to an unknown source

The risk of HBV transmission after an unknown source exposure in the UK is very low. It will depend on the prevalence of HBsAg and HBeAg in the population. This

† An anti-HBs level of 100miU/ml is considered to reflect an adequate response to the vaccine and to confer protective immunity. Levels of anti-HBs between 10 and 100 miU/ml may indicate a response to the vaccine but one that may not necessarily confer immunity and may require boosting.

Table 4 Dosage of hepatitis B immunoglobulin

| Age in years | HBIG dose |
|---|---|
| 0 – 4 | 200 IU |
| 5 – 9 | 300 IU |
| 10 or more | 500 IU |

will vary with locality and might be expected to be higher in inner city areas. Seroprevalence surveys of women attending antenatal clinics in inner London have found an HBsAg prevalence of 0.5% to 1%[4,5]; whereas 4% of homosexual male attenders at a London genitourinary medicine clinic were HBsAg positive[6].

Although the needles involved in community needlestick injuries are often believed to have been discarded by injecting drug users, this will not always be so. Furthermore, not all injecting drug users are HBsAg positive. A recent (and continuing) survey of the prevalence of anti-HBc (a marker for present and previous HBV infection) in the saliva of injecting drug users in England and Wales in 1991 found that only 427 (31%) of 1375 tested had detectable anti-HBc[7]. If it is assumed that all these infections were acquired in adolescence or adulthood, that 10% of those infected in adult life will develop persistent HBV infection and remain HBsAg positive, and that 20% of these will also be HBeAg positive, then the likely prevalence of HBeAg in such injecting drug users is about 1 in 160.

CDSC has never, to date, received a convincing report of HBV transmission having occurred after an unknown source needlestick exposure and it is suggested that HBIG has little part to play following such incidents. The risk of HBV transmission after percutaneous exposure to HBeAg positive blood has been estimated to be 30%; HBV transmission might thus be expected to occur in around 1 in 500 unknown source percutaneous exposures. Trials of the use of hepatitis B vaccine, with and without HBIG, for the prevention of transmission of HBV from HBeAg positive mothers to their babies (where, without intervention, the risk of transmission is over 80%) suggested that hepatitis B vaccine alone prevented between 70% and 85% of infections and that the addition of HBIG to the regimen conferred protection on an extra 10 - 15% of infants[8]. If it is assumed that hepatitis B vaccine given without HBIG will similarly prevent 70 - 85% of possible infections after percutaneous exposure, and that the use of HBIG in addition would prevent a further 12.5%, then HBIG could be expected to prevent HBV transmission in around 1 in 4000 (ie, 500 x 100/12.5) unknown source percutaneous exposures.

The present cost (excluding administrative costs), of an adult dose (500iU) of HBIG is £93. The estimated cost of giving HBIG in addition to hepatitis B vaccine, to prevent a single case of HBV infection as a result of an unknown source percutaneous exposure, is of the order of £400,000. Corresponding estimates of the cost of preventing a case of persistent HBV infection and death from acute hepatitis B are £4 million and £40 million, respectively. These estimates are conservative, because they leave aside considerations of the frequency with which discarded needles are contaminated with blood and the rate of decay of HBV infectivity in the environment. It is suggested, therefore, that HBIG should be used after unknown source exposures only in those known to be non-responders to hepatitis B vaccine. These individuals should be offered a single dose of HBIG within 24 hours of reporting the incident (and may be offered a booster dose of hepatitis B vaccine at the same time).

Exposed persons with no history of hepatitis B vaccination, and those who have previously received only one dose of the vaccine, should be offered an accelerated course of hepatitis B vaccine (doses spaced at 0, 1 and 2 months with a booster dose at 12 months for those at continuing risk of exposure to HBV). Those who have previously received two or more doses of hepatitis B vaccine, but are of unknown HBV status, should be offered a single dose of the vaccine. Known responders to hepatitis B vaccine will not require prophylaxis after unknown source exposure incidents, though the occasion may provide an opportunity to give a 'routine' booster dose of HB vaccine.

### (iii) After exposure to an HBsAg negative source or after non-significant exposure

Although specific HBV prophylaxis will not be required for the incident itself, exposed persons who have not previously received hepatitis B vaccine and who are thought to be at continuing risk of exposure to HBV should start a course of vaccine. Those who have received part of a course should complete it as originally planned.

## Follow-up of exposed persons

Serological follow-up of exposed persons is important for several reasons. Firstly, the immune response to primary courses of hepatitis B vaccine should be documented in those at occupational risk of HBV infection; those who do not develop ≥10miU/ml anti-HBs after vaccination may benefit from additional doses of hepatitis B vaccine. Secondly, post-exposure HBV prophylaxis may fail, but the resulting HBV infection may be subclinical and detectable only by serological testing. Finally, exposures which carry a risk of HBV infection may pose a risk of transmission of other blood borne viruses, for which serological follow-up may be appropriate. A follow-up blood specimen should therefore be obtained from exposed persons six months after the exposure incident, and serum stored for a minimum of eighteen months.

Follow-up specimens from all exposed persons at continuing risk of HBV exposure, except those who were known to be responders to hepatitis B vaccine at the time of the incident and those whose post-exposure prophylaxis consisted of HBIG alone, should be tested for anti-HBs. Where anti-HBs is not detectable, the specimen should be tested for anti-HBc (and HBsAg if appropriate) in parallel with serum stored from the initial post-exposure specimen. Follow-up specimens from exposed persons given HBIG but not hepatitis B vaccine post-exposure should be tested for anti-HBc and HBsAg in parallel with the initial post-exposure specimen.

Any exposed person developing an illness compatible with a diagnosis of acute hepatitis in the six months after the exposure incident should have appropriate diagnostic tests performed at that time. HBV prophylaxis should be considered for sexual and other close contacts of any exposed person found to be HBsAg positive. Persons who become anti-HBc or HBsAg positive as a result of an exposure incident should be reported to CDSC.

## Supplies of HBIG

All public health laboratories hold stocks of adult doses (500iU) of HBIG. Supplies may also be obtained from the Hepatitis Section, CDSC, where 200iU dose vials are also held (telephone 081 200 6868 ext 3404 or 3405, or out of hours via the duty doctor).

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Report prepared by: *J Heptonstall, Hepatitis Section, PHLS Communicable Disease Surveillance Centre.*

# The laboratory diagnosis of mycobacterial disease

*P A Jenkins*


## Summary

The diagnosis of mycobacterial disease still relies heavily on conventional microscopy and culture techniques. More modern methods, such as the Bactec 460 radiometric system and the Roche biphasic system, are becoming available but are not yet widely used. Non-culture methods eg, using molecular biology techniques or gas liquid chromatography, are being developed but are currently the province of research and reference laboratories. Disease caused by species other than *Mycobacterium tuberculosis* poses problems of diagnosis and treatment. These other species can be identified by comparatively simple techniques but, as they are widely distributed in the environment, deciding between casual contamination and true infection can be difficult. Although such infections are not a major problem in numerical terms, the variable response of patients to treatment means that conventional sensitivity testing is of little assistance, and it is necessary to seek evidence of synergy in drug combinations for the effective treatment of these infections.


## Introduction

Mycobacterial infection has been the subject of much recent atttention[1-4]. Articles have highlighted the need for prompt identification and treatment of cases of tuberculosis (particularly those who are smear positive), the difficulty of diagnosing disease in children, the increase in incidence of disease associated with HIV infection, and the problem of treating infections due to opportunistic mycobacteria. The recognition of these problems and that tuberculosis is far from being eradicated has rekindled interest in the disease.

## Diagnostic methods

Direct microscopy of sputum using a Ziehl-Neelsen or fluorescent stain remains the simplest and quickest method of detecting the truly infectious patient ie, the one with open pulmonary tuberculosis. It is much less useful in non-pulmonary disease and childhood disease; in these cases, it is necessary to rely on culture, which traditionally uses a solid medium – either egg-based Lowenstein Jensen or agar-based Middlebrook 7H 10, both of which can require incubation for six to eight weeks before growth is evident[5].

### Culture techniques

Most mycobacteria grow more quickly in liquid, and Kirchner's medium has been used for many years for this method. It is important to recognise when growth has occurred, as subculture onto solid medium is required for identification and sensitivity testing. This has been facilitated by the Bactec 460 radiometric system (Becton and Dickinson, Oxford), which uses a Middlebrook 7H 12 broth containing a $C^{14}$ labelled substrate. When metabolised, $C^{14}$ labelled $CO_2$ is produced and is measured and displayed as a growth index by the apparatus. Growth of *Mycobacterium tuberculosis* occurs in seven to ten days and a sensitivity result can be available in another five days. However, the system is expensive and the use of radioactive labels poses problems for some laboratories[6]. A new medium, Bactec 13A, has recently been introduced which is specifically designed to accept up to 5ml of blood or a bone marrow specimen. It is particularly suitable for blood cultures from AIDS patients when a disseminated infection with *M. avium* is suspected[7].

For laboratories unable to use the Bactec system, Roche have introduced the MB Check culture system. This is a biphasic system combining a primary liquid phase in the same bottle as a solid agar-based phase. The latter incorporates both chocolate agar, to detect contamination, and a phase containing NAP (p-nitro-α-acetylamino-β-hydroxy propio-phenone), which can distinguish between organisms of the tuberculosis complex and other mycobacteria. The final part of the solid phase is a Middlebrook

**Table 1 The opportunistic mycobacteria that most commonly infect man**

| Species | Pulmonary disease | Cervical lymphadenopathy | Localised abscesses and skin infections |
|---|---|---|---|
| *M. avium** | • | • | |
| *M. chelonei* | | | • |
| *M. fortuitum* | | | • |
| *M. intracellulare* | • | • | |
| *M. kansasii** | • | | • |
| *M. malmoense* | • | • | |
| *M. marinum* | | | • |
| *M. scrofulaceum* | • | • | |
| *M. xenopi* | • | | |

* *M. avium* may be associated with disseminated infections, and *M. kansasii* with bone/joint/tendon infections, but these are extremely rare.

7H 10 agar from which subcultures can be made for further identification and sensitivity tests[8].

Both the Bactec and Roche systems are expensive and less relevant to the needs of developing countries, where a balance has to be struck between labour and consumable costs when deciding which system to use. The safety aspects of liquid cultures also have to be taken into consideration and weighed against a reduction in the time taken to produce a result.

### Other techniques

A non-cultural method of diagnosing mycobacterial infection has been sought almost since Robert Koch first isolated the tubercle bacillus in 1882. None of the generally accepted serological tests has been adopted because of a lack of sensitivity and specificity[9]. It was hoped that DNA probes would be the answer but so far these have proved no more sensitive for diagnosis with primary specimens than direct microscopy. They are of use, however, in the rapid identification of mycobacterial strains, and probes are now available for a number of different species[10]. The original probes used a radioactive marker and had a very restricted shelf-life. Currently available probes rely on a bioluminescent marker and last much longer. They also require only a relatively simple luminometer instead of a gamma-counter. They are particularly useful for testing Bactec vials when the growth index is approximately 300, a situation which can be achieved in four or five days with tubercule bacilli or 24 hours with the *Avium-intracellulare* complex (Accuprobe Gen-Probe Inc. Ca, USA).

The most important development has been the use of the polymerase chain reaction to amplify the DNA from as little as three bacilli in a specimen to detectable amounts[11]. This is potentially the most powerful diagnostic tool available but currently remains the province of research laboratories. It requires extreme care to avoid contamination with foreign DNA and inhibitors in specimens can halt the action of the Taq enzyme, which is essential for the amplification process. It will be some years before the test is routinely available, even in reference laboratories.

Another area receiving attention is the detection in specimens of specific substances that are only present in association with mycobacteria. This requires the use of gas liquid chromatography (GLC) linked with mass spectrometry or high pressure liquid chromatography (HPLC). Both systems are expensive and access to them is limited. They rely on the detection of tuberculostearic acid (10 methyloctadecanoic acid), mycocerosic acids, 2-alkanols, phthiocerols or mycolic acids. This is still a specialist area requiring expert knowledge and the system is very much a research tool[12]. Mycobacterial antigens have been detected in CSF specimens using rabbit antisera to BCG in an ELISA system[13] – this technique, however, requires further evaluation.

These approaches are mainly directed towards the primary diagnosis of classical tuberculosis. The laboratory diagnosis of disease due to mycobacteria other than *M. tuberculosis*, or opportunistic mycobacteria, relies totally on culture of the organism and its subsequent identification by classical cultural and biochemical methods. DNA probes, and detection of specific substances by thin layer chromatography GLC or HPLC, are being used but are restricted to reference laboratories.

## Opportunistic mycobacteria

The species that most commonly cause human disease are listed in table 1. Pulmonary disease in adults and localised lymphadenopathy in children are the commonest presentations. Soft tissue infections and local abscesses can be caused by *M. fortuitum*, *M. chelonei* and *M. kansasii*, and superficial skin infections by *M. marinum* (fish-tank granuloma). Other species such as *M. gordonae* and *M. terrae* can cause disease but cases are extremely rare[14]. The identification of these organisms, so far as is necessary for the effective treatment of the patient, is reasonably straightforward. The system developed by Marks[15] uses a small number of simple tests such as temperature range, oxygen preference, pigmentation and Tween hydrolysis.

The true incidence of infection with opportunistic mycobacteria is difficult to determine. Tuberculosis is a statutorily notifiable disease but other mycobacterial infections are not. Laboratories are encouraged to report all new cases of disease due to opportunistic mycobacteria to the PHLS Communicable Disease Surveillance Centre. However, not all laboratories do this and, furthermore, there is no strict definition of what constitutes a 'genuine' case. All opportunistic mycobacteria are environmental organisms and can easily gain access to clinical specimens. Single isolates from sputum, for example, are unlikely to be clinically significant and even multiple isolates may be the result of colonisation of damaged lung tissue and not genuine infection.

New cases of infection due to *M. kansasii* and *M. xenopi* have remained steady at approximately 30 and 20 per year, respectively, but there has been an increase in those due to the MAIS complex (*M. avium*, *M. intracellulare* and *M.*

*scrofulaceum*), rising from 33 in 1985 to 77 in 1990. This is attributable to disease in patients infected with the human immunodeficiency virus (HIV). In the UK, HIV-infected patients are more likely to develop disease due to the MAIS complex rather than due to the reactivation of dormant tubercle bacilli, as few of those currently presenting with HIV infection harbour tubercle bacilli from an earlier infection[16]. The most common strains recovered from these patients are those of *M. avium*, rather than *M. intracellulare*. The reported incidence of MAIS infection is thought to be a considerable underestimate, partly due to the confidentiality surrounding HIV infection.

The other opportunistic mycobacterium of importance is *M. malmoense*. This was first described in 1977 and was then thought to be of little consequence[17]. Seventeen cases of pulmonary disease and five cases of cervical lymphadenopathy in children are known to have occurred between 1953 and 1981. During the 1980s the incidence rose steadily to about 35 new cases of pulmonary diseases per year and up to 19 cases of cervical lymphadenopathy. The identification of *M. malmoense* relies on the presence of a specific lipid pattern detectable by thin-layer chromatography[18].

A recent problem has been the contamination of bronchial washings with *M. chelonei* following bronchoscopy[19]. This organism is a non-pigmented, rapidly growing, psychrophile (ie, it grows better at 25°C than 37°C) which can cause localised abscesses but is most frequently present as an environmental contaminant. Its presence in bronchial washings has been due to the practice of rinsing fibre optic bronchoscopes with tap water instead of sterile distilled water.

## Drug sensitivity tests

Determination of the sensitivity of *M. tuberculosis* to the drugs used in treatment is well established and there is a good correlation between *in vitro* sensitivity and *in vivo* response. This is not true for opportunistic mycobacteria but, despite this, the system used for *M. tuberculosis* has been applied to them. *M. kansasii* is sensitive *in vitro* to rifampicin and ethambutol and a nine month regimen of these two drugs appears to be effective (British Thoracic Society – personal communication). However, the situation with the MAIS complex, *M. malmoense* and *M. xenopi* is more complicated. These organisms show varying degrees of resistance to the usual anti-tuberculosis drugs, but retrospective studies have indicated that a significant majority of patients do respond to standard anti-tuberculosis chemotherapy provided it is given for two years[20,21].

There is some evidence of synergy between ethambutol and rifampicin and also streptomycin. However, current work at the Mycobacterium Reference Unit is showing that, although this may apply to the MAIS complex, it does not apply to *M. malmoense*. This species has an optimum pH of about 6.0 and most sensitivity studies have been carried out at about pH 7.0. The suboptimal pH increases the apparent sensitivity of the organism and produces spurious results.

The incidence of classical tuberculosis is likely to decline in the long term in the UK but certain groups eg, the elderly and immigrants from the Indian subcontinent, will continue to be at higher risk for some years. The chemotherapy of opportunistic mycobacterial infections requires more investigation. Exposure to environmental mycobacteria is unavoidable and infections will continue to occur.

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# Leprosy surveillance

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## England and Wales

Leprosy became a notifiable disease in 1951 and 1507 notifications were recorded in England and Wales up to December 1990. During this time, 223 duplicates and 25 incorrect diagnoses were identified, leaving a total of 1259 registered cases at the end of 1990. Of these, 840 were males, 395 were females, and in 24 cases the sex was not recorded. The ages of these cases ranged from 3 to 86 years (mean 34 years). The commonest age group for notification was 20-29 years. The most common ethnic groups were those from the Indian subcontinent: Indians, Pakistanis and Bangladeshis comprised one third of the total cases reported. No indigenously acquired cases of leprosy have been reported in England and Wales since notification began 40 years ago.

Nine hundred and forty-five cases have left the register since notifications began, because their leprosy has been cured (482 cases); they have left the country (303) or they have died (135). Ninety-six cases have been lost to follow-up. Figure 1 shows the clinical type of leprosy for the 243 cases that remain on the register. The number of cases of borderline and tuberculoid leprosy has increased in the last decade. The clinical features recorded include anaesthesia (44%), deformity (22%), ulceration (15%), absorption or amputation (0.1%) and visual impairment or blindness (0.05%). The number of new registrations has declined steadily since reaching a peak in 1964[1], averaging 12 per year for the past five years (1986 – 11; 1987 – 11; 1988 – 13; 1989 – 18; 1990 – 9).

The ages of the nine new cases notified in 1990 ranged from 13 to 67 years, with a mean of 37 years. There were eight males and one female. Their leprosy was lepromatous in one case, borderline in four, tuberculoid in one and indeterminate in two; the type was not recorded for one patient. Five were receiving chemotherapy and four were under surveillance alone. A total of 175 patients remained on therapy, consisting of dapsone in 31%, dapsone with rifampicin in 14%, triple therapy (dapsone, rifampicin and clofazimine) in 23%, and other agents or combinations in 32% of cases.

## The global situation

Leprosy continues to be a major public health problem in Africa, Asia, and Latin America but the global estimate has fallen from about 10 million cases a decade ago to about 5 million in 1991[2]. This improvement has been due largely to the introduction of multidrug therapy (MDT) in the early 1980s. MDT coverage continues to increase in many countries and the World Health Organisation (WHO) is committed to the global elimination of leprosy as a public health problem by the year 2000, with elimination defined as the attainment of a prevalence of less than one case per 10,000 population. The current prevalences of estimated and registered cases for the six WHO regions are given in table 1[3].

**Figure 1 Cases in England and Wales by year of notification and clinical type**

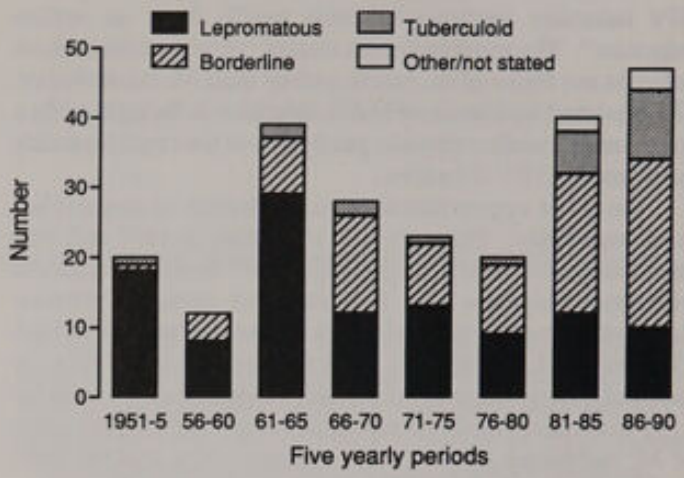


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**Table 1 Estimated prevalence rates by WHO region[3]**

| WHO region | Population (millions) | Estimated cases | | Registered cases | |
|---|---|---|---|---|---|
| | | Number | Prevalence rate per 10 000 | Number | Prevalence rate per 10 000 |
| Africa | 535 | 96 000 | 17.1 | 352 222 | 6.6 |
| Americas | 735 | 391 000 | 5.3 | 335 490 | 4.6 |
| Eastern Mediterranean | 400 | 207 000 | 5.2 | 92 606 | 2.3 |
| South-East Asia | 1 341 | 3 750 000 | 28.0 | 2 190 324 | 16.3 |
| Western Pacific | 1 522 | 238 000 | 1.6 | 110 125 | 0.7 |
| Europe | 852 | 9 000 | 0.1 | 7 021 | 0.1 |
| Total | 5 381 | 5 511 000 | 10.2 | 3 087 788 | 5.7 |

# Food safety: the HACCP approach to hazard control

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## Summary

The Hazard Analysis Critical Control Point (HACCP) approach is becoming increasingly recognised as a valuable means of identifying and controlling hazards in the food production process, and thereby ensuring that food reaching the consumer is safe. The central feature of HACCP analysis is the determination of critical control points (CCPs) – those stages in the process which must be controlled to ensure the safety of the product. Once identified, a monitoring system is set up for each CCP to ensure that correct procedures are maintained and actions taken if CCP criteria are not achieved. The chief advantage of HACCP is that it is proactive; it aims to prevent problems occurring. Although HACCP is most readily applied to manufacturing processes, attempts are being made to adapt the system to other sectors of the food industry, such as catering.

## Introduction

The consumer expects to be able to purchase and eat food which is safe and has been handled hygienically during harvesting or slaughter, processing, preparation and sale. The control of food safety during all stages of the production process is a balance of self-imposed regulation by the industry and controls exerted by the food law enforcement authorities. Controls need to be preventive so that contaminated or otherwise unsafe food does not reach the consumer. Industry and governments around the world are examining systematic methods of achieving this.

One such system, Hazard Analysis Critical Control Point (HACCP) is becoming increasingly recognised by governments and the food industry as a valuable preventive and proactive system. The system was originally developed in the 1960s by the Pillsbury Company, United States Army laboratories at Natick, and the National Aeronautics and Space Administration (NASA) in a collaborative effort to develop safe foods for astronauts involved in the United States space programme. The Pillsbury Company presented the HACCP concept to the American National Conference for Food Protection in 1971 and the system has since been modified and developed by the food industry.

HACCP has received national and international recognition. The International Commission on Microbiological Specifications for Foods commended HACCP to the food industry[1]. In the United Kingdom, the report to the Government on the Microbiological Safety of Food (Chairman – Sir Mark Richmond)[2] made frequent reference to HACCP and included examples of HACCP applied to certain processes. The report recommended "that all food processes should be designed on HACCP principles" and suggested that enforcement officers should encourage industry to adopt the HACCP approach. The Codex Alimentarius Commission, established in the 1960s by the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) to develop internationally agreed codes, has drawn up an HACCP code through an *ad hoc* group of its Food Hygiene Committee[3]. The code is an attempt to draw together the developments of HACCP over the past 20 years into an internationally agreed set of principles.

## What is HACCP?

Hazard Analysis Critical Control Point (HACCP) is a systematic approach to the control of potential hazards in a food operation. A hazard is anything that could harm the consumer; it may be biological, chemical or physical. HACCP aims to identify problems before they occur, and establish mechanisms for their control at the stages in production critical to ensuring the safety of food. Control is proactive, since the identification of potential hazards and preventive measures, and the establishment of monitoring and remedial actions in advance, means that the hazard does not occur. Table 1 lists the seven principles of HACCP as set out in the Codex Alimentarius Commission code[3].

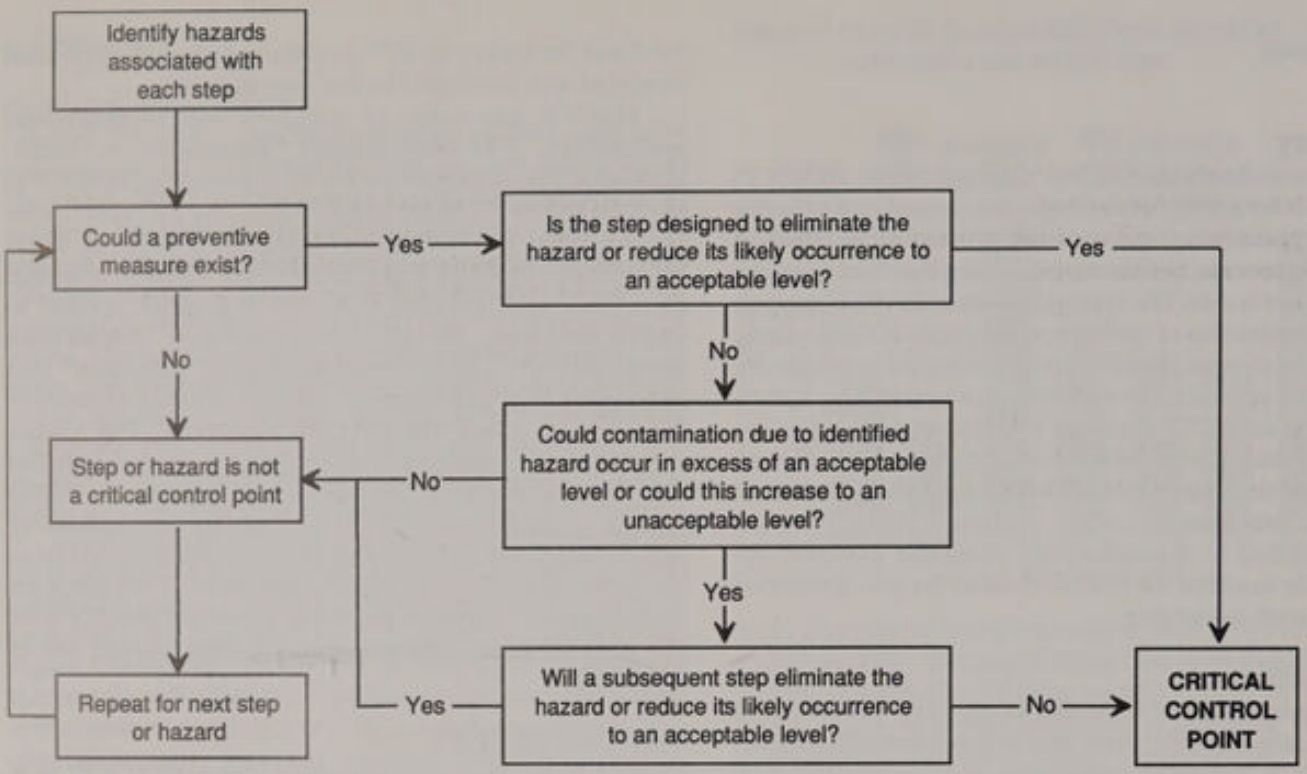
In analysing the food operation, consideration must be given to the type of raw materials and ingredients used in the process, the means available to control hazards, the likely use of the product once it is manufactured or served and the population at risk, including any epidemiological evidence relating to the safety of that product.

**Table 1 Seven principles of HACCP***

1. Identify the potential hazards associated with food production at all stages up to the point of consumption. Assess the likelihood of occurrence of the hazards and identify the preventive measures necessary for their control.
2. Determine the points, procedures and operational steps (**critical control points** – CCPs) that can be controlled to eliminate the hazards or minimise their likelihood of occurrence. A 'step' means a stage in food production or manufacture eg, the receipt or production of raw materials, harvesting, transport, formulation, processing and storage.
3. Establish target levels and tolerances which must be met to ensure the CCP is under control.
4. Establish a monitoring system to ensure control of the CCP by scheduled testing or observation.
5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
6. Establish procedures for verification, including supplementary tests and procedures to confirm that HACCP is working effectively.
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

* Adapted from reference 3.

Figure 1 The HACCP decision tree*




* Adapted from reference 3.

## The HACCP logic sequence

The HACCP logic sequence shows the steps required to carry out an HACCP procedure. A team is set up, consisting of technical and non-technical personnel. The product to be analysed is described and its intended use identified. An example would be a chicken product intended for consumption by the public. The flow-chart of its production is developed, and then verified by 'walking' the production area. Any alterations can be made at this stage and the flow-chart agreed. The potential hazards are then listed against each step; there may be several different hazards for any one step. Preventive measures can then be listed against each hazard. In the case of chicken, a potential hazard would be the survival of salmonellas during cooking; the preventive measure would be to ensure thorough cooking and heat penetration of the carcase.

The crux of the HACCP analysis is the determination of critical control points (CCPs). By applying the HACCP decision tree (Figure 1) the team can 'home in' on those steps which are critical to the safety of the product; set targets and tolerances for that CCP, and determine how, when and by whom the CCP is to be measured and observed. The target level is the value to be achieved at each CCP and the tolerances give the acceptable variation from that target. The target level for cooking chicken might be to heat until deep parts of the flesh reach 75° C; the tolerance could be -2° C from the target. The variables to be monitored should have a measurable value, preferably one which is quick to assess, such as temperature, time or a visual inspection-colour change. In addition, the team needs to develop instructions and procedures for dealing with deviations from the CCP tolerance values, including instructions to reprocess or dispose of the products. A system for monitoring each CCP should then be established, with appropriate record keeping and documentation. Table 2 illustrates part of a hazard analysis of the production of powdered formula milk[4] and also shows how HACCP can help identify future improvements to control hazards.

## Evaluation of HACCP

The traditional approach to food safety has involved the training of food handlers, inspection of premises and end product testing by microbiological analysis of samples taken from batches or a day's production. Training and inspection remain key elements of food safety, and there are new developments to increase their effectiveness[5]. However, there are a number of disadvantages associated with end product testing:

(i) it is retrospective; the product is already made and test results may not be available for several days

(ii) it may involve product recall if a problem is highlighted; this can only be partly effective and is usually costly

(iii) it may fail to detect contaminated batches, and as it is possible to sample only a small number of units from a batch for economic reasons, unsafe units may be missed leading to the false assumption that the whole batch is safe[6]

(iv) it is expensive; expertise and facilities are needed to carry out tests

(v) it only involves a limited number of staff in hazard control.

HACCP has a number of advantages over end product testing:

(i) HACCP is proactive and preventive; prescribed remedial action can be taken quickly before problems occur

(ii) it may identify hazards that have not been experienced; it is therefore particularly useful when setting up new operations

(iii) it applies to all parts of the process rather than to samples selected for testing

(iv) it allows resources to be concentrated on critical control points rather than being spread thinly across the whole process

(v) it involves all levels of staff, not just technical personnel, and is controlled by those involved directly in production rather than microbiologists in remote laboratories.

There are, however, limitations associated with HACCP. HACCP is only likely to be successful where the principles of good hygienic practice are already in place. If the structure, equipment and cleaning standards are inappropriate, the development of an HACCP system will be difficult. An understanding of what constitutes a hazard, and of the appropriateness of preventive measures, is necessary for the system to be effective. A comprehensive HACCP system lends itself primarily to manufacturing operations but the principles of identifying hazards and introducing suitable control measures can be applied to any food operation.

## Recent government initiatives

Officials in the Department of Health (DOH) and the Ministry of Agriculture, Food and Fisheries (MAFF) have taken a leading role, within the FAO/WHO Codex Alimentarius Commission, in the development of an internationally agreed definition of HACCP and its applications[3]. In addition, the government has promoted the use of HACCP through the release of a leaflet[7] which has been supplied to environmental health departments for distribution to food businesses. Technical papers, giving more detailed information about HACCP, have been provided to environmental health departments, and other articles on the subject have been published[8].

The DOH and MAFF have also been involved in the training of environmental health officers in aspects of HACCP. More than 20 workshops have been held advising officers of the basic principles of HACCP and how these can be applied in normal inspection routines. In addition, two major conferences have taken place which were targeted at the food industry, enforcement authorities and medical

**Table 2 Selected critical control points in the production of powdered formula milk***

| | Critical control point | | | |
|---|---|---|---|---|
| | Concentration of vitamin enriched milk | Mixing of oil and vitamin | Transportation of product | Packaging of product |
| Description | Two stage evaporator (stage 1: 60° - 70°C; stage 2: 50° - 60°C). | Materials mixed in a tank at 20°C. | By air through ducting into storage tanks. | In an impermeable laminate sachet. |
| Hazards | Drop in temperature due to halt in production. Bacteria may grow in held product. | Raw materials may be contaminated if incorrectly handled. | Contamination carried in cooling air. Cracks in ducting may allow contamination. | Laminate may be contaminated. Air supply to filler may be contaminated. Residue in filler could contaminate fresh product. |
| Preventive measures | Ensure correct feed to evaporator. Ensure correct temperature for both stages. | Supplier's quality assurance of raw materials. | Filter air. Inspect ducting for leaks. | Discard outer layer of laminate on spool. Filter air. Dry-clean filler to prevent residue build-up. |
| Target/tolerance levels | Second stage minimum temperature 48°C. | Tested product is free of pathogens. | Class 1 filters on cooling air inlets. No leaks in ducting. | First four metres of spool discarded. Class 1 filters on air supply to filler. |
| Action in event of deviation from target/tolerance level | Quarantine product. | | Production halted if leaks discovered. | |
| Planned improvements | Introduction of new equipment to avoid halts in production. | | | Consider ultraviolet treatment to disinfect laminate. |

* Adapted from reference 4.

professionals involved in food safety; further seminars are planned in England and Scotland.

## Food hygiene inspections

Under Section 40 of the Food Safety Act 1990[9], various codes of practice advising on the application of legislation have been issued to enforcement authorities by the government. These include Code of Practice No. 9 on Food Hygiene Inspections which outlines the steps an environmental health officer should take when carrying out a food hygiene inspection[10]. It advises environmental health departments to prioritise visits according to relative risk and to take into account whether the food business has an effective management system in place. The Code draws the environmental health officer's attention to those parts of the operation which are critical to the safety of food. It advises that an inspection should include a preliminary assessment of the food safety hazards associated with the operation to identify areas requiring closer scrutiny. Where hazard analysis systems are already fully developed, the inspection approach should be modified; particular attention should be paid to determining whether the necessary monitoring and verification of CCPs is being carried out. Many food businesses, particularly small ones, may not have formal hazard analysis systems. In such cases the environmental health officer should have special regard to the hazards associated with the business.

There is no legal requirement to introduce HACCP, although some organisations see an effective HACCP system as part of the development of a 'due diligence' defence under Section 21 of the Food Safety Act 1990[9].

## Future developments

HACCP systems are being taken up increasingly by the food industry. HACCP is mentioned in European Commission directives, in particular the proposed Food Hygiene Directive[11] and the Meat Products Directive[12]. In the United States, HACCP is already established in principle in some national legislation and has been promoted rigorously by the US government food agencies, including a recent programme aimed at the fish and shellfish industries. Conferences have been held around the world advising that HACCP should be operating in food manufacturing organisations which wish to import into the United States avoiding extensive dockside checks. There is no reason why such control of imports may not spread into other commodity sectors, and to other countries.

Although HACCP has been used primarily in food manufacturing situations, the principles of identifying hazards and suitable controls can be applied to other sectors of the food industry, eg, catering. Some large cook-chill and cook-freeze production units have been able to adopt HACCP systems, as these processes lend themselves to this type of systematic analysis. The DOH guidance on cook-chill and cook-freeze advises that HACCP should be used as a method to control hazards[13]. However, traditional catering operations have had greater difficulty in applying HACCP, partly due to the wide range of foods being processed. One of the problems that this industry faces is a general lack of technical expertise, particularly in the field of microbiology, to which caterers can turn. Many caterers may have difficulty in identifying hazards and effective controls. With this in mind, a catering industry group at Campden Food and Drink Research Association has been working with DOH and MAFF officials to develop a system suitable for catering, which identifies hazards applicable to any catering operation and provides 'generic CCPs' for the caterer. Another part of the food industry that has had difficulty in adopting the complete HACCP system has been the small retailing sector, such as small butchers and cornershops. A similar type of approach may be applied to these operations in the future.

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