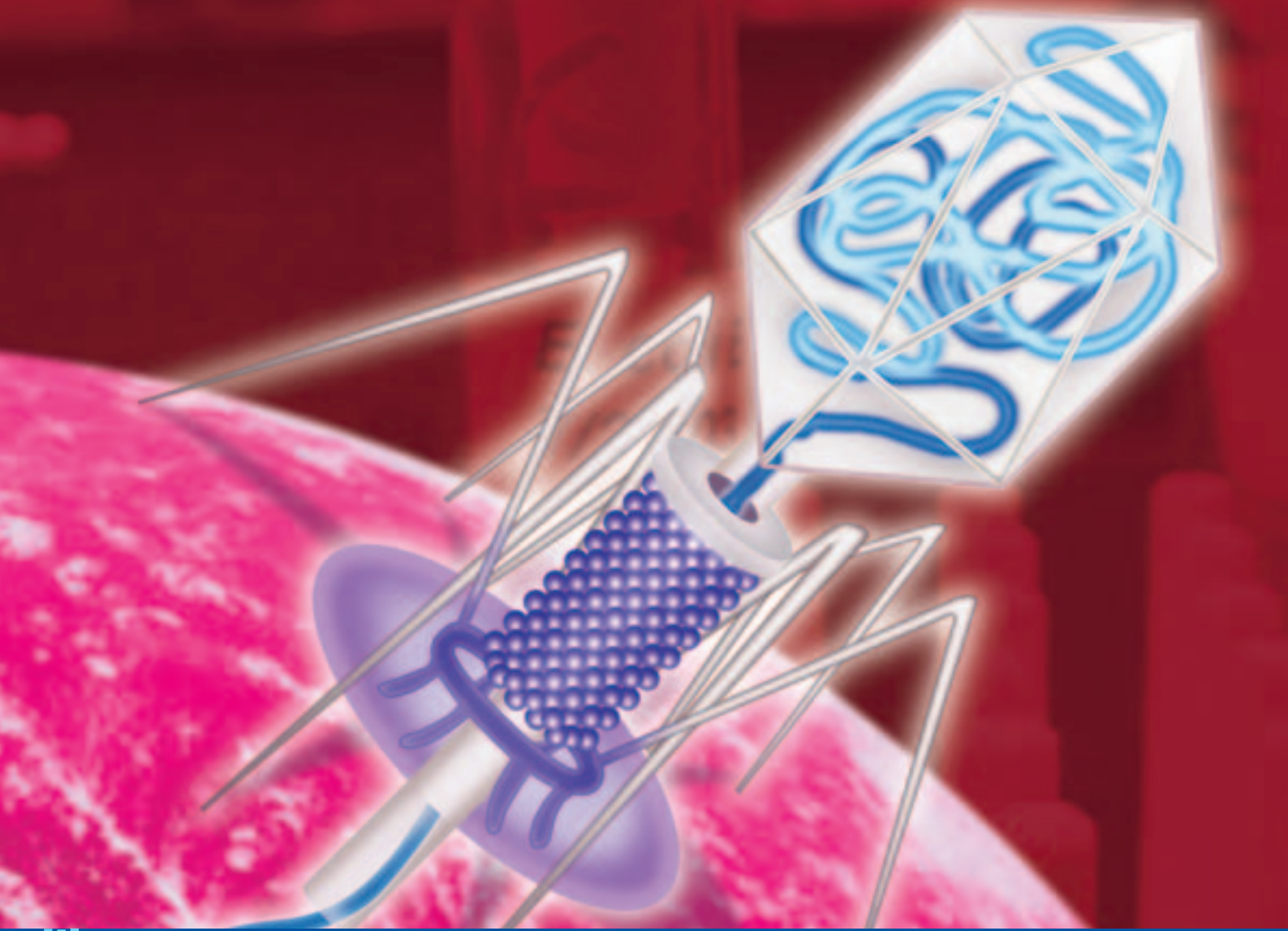
Microbiologist

The magazine of the Society for Applied Microbiology ■ March 2007 ■ Vol 8 No 1 ISSN 1479-2699

BACTERIOPHAGE THERAPY

Geoff Hanlon discusses antibiotic resistance as a driver for exploring alternative therapies and revisits the potential of **bacteriophage therapy**



INSIDE

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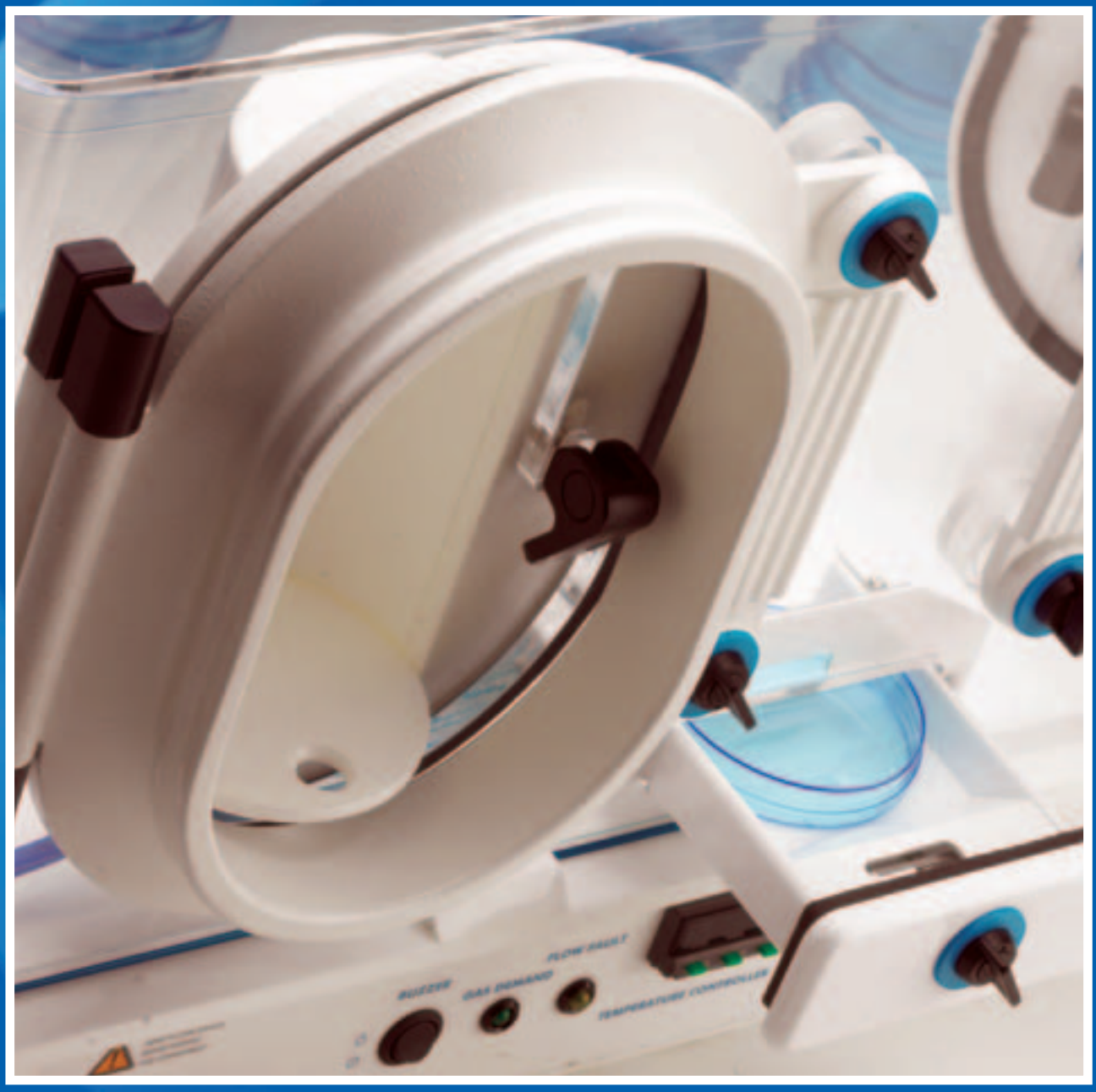

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Microbiologist the magazine of the Society for Applied Microbiology

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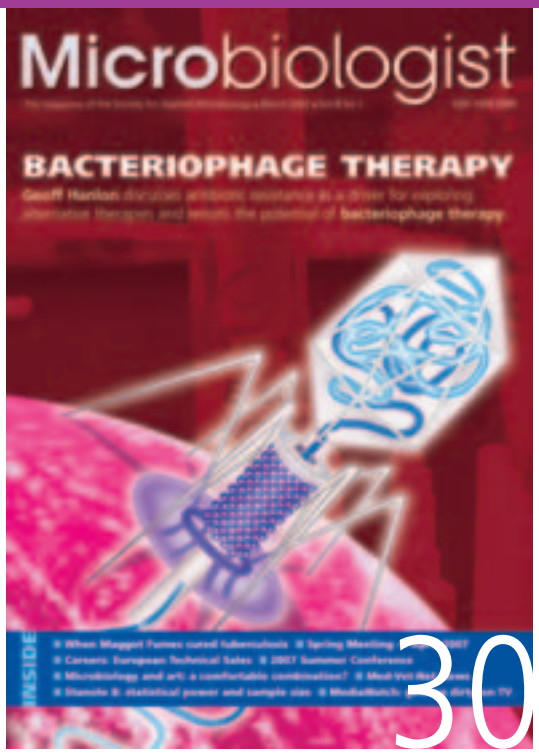

Bacteriophage Therapy

Spring Meeting 11 April 2007

When maggot fumes cured tuberculosis

information

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Cover illustration, 'Bacteriophage therapy': Stephen Pollard

editorial

Lucy Harper talks about antibiotic resistance and asks whether we should be looking at alternative therapies

While I was researching this editorial, an interesting piece of news hit the headlines: there has been confirmation of the link between the use of antibiotics and the development of resistance.

In this study, published in the *Lancet* on 10 February 2007, (http://www.thelancet.com/ journals/ lancet/article/PIIS0140673607602359/abstract) Prof. Herman Goossens and colleagues of the University of Antwerp, Belgium, found that streptococci in the mouths of healthy volunteers given either azithromycin or clarithromycin (macrolides), developed resistance to macrolides when compared to a control group who had taken a placebo over the same time period. Infact, there was no resistance among those taking the placebo.

Goossens *et al* also found that the oral microflora was affected by the macrolides and this effect lasted for up to 180 days after taking the drugs.

The association between antibiotic use and resistance of microorganisms is a known phenomenon, but until now a causal effect has not been shown. This study highlights the need for awareness of the ecological implications, and the requirement of prudence in prescribing antibiotics in a healthcare setting.

There are a number of 'alternative' therapies which have antimicrobial properties but which don't appear to contribute to the development of antibiotic resistance. Perhaps we should be looking at these as possible alternatives, or at least adjuncts to lower dose antibiotic therapy. For example, the ingestion of cranberry juice and the berries themselves has shown to have prophylactic effects against urinary tract infection (UTI), possibly by preventing bacterial adherence to host cell membranes. Other 'alternative' remedies include garlic and tea tree oil which have antibacterial, and also antifungal, and antiviral activity.

This issue of *Microbiologist* looks at antibiotic resistance as a driver for exploring alternative therapies: revisiting the potential of bacteriophage therapy (see page 30 for full article). A long-forgotten form of treatment in the West during the antibiotic era, Bacteriophages or 'bacteria eaters' could be one step towards finding a balance between antibiotics and alternative therapy in tackling infectious disease without encouraging the development of antibiotic resistance. You can find out the latest developments in Bacheriophage therapy at our Spring meeting in Manchester on 11 April 2007. The full programme for this meeting is available on page 24 and online at www.sfam.org.uk/spring_meetings.php.

Our second feature article looks at alternative therapies from a more historical perspective — maggot fumes in the treatment of Tuberculosis (TB). A fascinating article which makes me glad that I wasn't in the Bradford area in the 1900s. The smell emanating from the 'maggotorium' must have been interesting to say the least and the benefits from treatment obviously far outweighed the awful stench that sufferers must have had to endure. Read more on page 33.

Our final feature article is far removed from the treatment of disease. This article looks at the much more aesthetically pleasing topic of microbiology and art. As well as the degradation of works of art by microbes, the creative aspects of combining art with science and the complex relationship between these two fields is discussed on page 36.

Finally, attentive readers will notice that this issue of *Microbiologist* has undergone a facelift. I hope that you will all benefit from the many improvements we have made to the layout and look of the magazine.

Lucy Harper

contribute

We are always looking for enthusiastic writers who wish to contribute articles to the magazine on their chosen microbiological subject.

For further information please email the editor, Lucy Harper at: **lucy@sfam.org.uk**

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Contributions: The editor is always looking for enthusiastic writers who wish to contribute articles on their chosen microbiological subject. For further information please email Lucy Harper at: **lucy@sfam.org.uk**

Disclaimer: The Society assumes no responsibility for the opinions expressed by contributors. The views expressed by Society officers and staff do not necessarily represent the official position of the Society.

Subscriptions: A subscription to *Microbiologist* is included in the annual SfAM membership fee. For further information about the many benefits of membership please see page 6.

Advertising: Information about advertising in *Microbiologist* and how to submit advertisements are can be found on the Society website.

Website: our website **(www.sfam.org.uk)** is a timely source of up-to-date information on all Society matters and maintains a comprehensive archive of articles and reports on a variety of microbiological topics.

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benefits

The Society for Applied Microbiology is the voice of applied microbiology within the UK and was founded in 1931. Society members play a leading role in shaping the future of applied microbiology, and enjoy many benefits, including:

- Substantially reduced rates for attendance at Society meetings and conferences
- Access to the members areas of the Society website
- Many generous grants and awards
- FREE access to three acclaimed journals

Detailed information about all these benefits and more can be found on the Society website at: www.sfam.org.uk

GRANTS & AWARDS: Many grants, awards and prizes are available to members including the W H Pierce Memorial Prize and Prizes for Student Oral Presentations and Posters at the Summer Conference. In addition to these substantial awards, the Society has funds to assist members in their careers as microbiologists. These include The President's Fund, Conference Studentships, Sponsored Lectures and the popular Students into Work Scheme.

Full details of all the Society's grants and awards can be found on the website together with PDF application forms available to download from the members area.

JOURNALS: The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. We also produce this quarterly colour magazine, *Microbiologist*, which contains features, topical news stories and full details of our meetings. The Society is also a partner with Blackwell Publishing in the monthly journal *Environmental Microbiology.*

Synergy is an online service provided by Blackwell Publishing that gives Full and Student Members FREE access to the online versions of the Society's three journals: *Journal of Applied Microbiology, Letters in Applied Microbiology* and *Environmental Microbiology*. Members can register for this service at http://www.blackwell-science.com. Members can also submit papers directly to our journals via an online submission service. For more information about Synergy or online manuscript submission, please visit the website.

MEETINGS: We hold two annual meetings. The January Meeting is a one-day meeting with parallel sessions on topical subjects. The Summer Conference is held every July and comprises a main symposium, a poster session, the AGM and a lively social programme. We also hold occasional joint ventures with other organisations on topics of mutual interest.

WEBSITE: The website is the best source of detailed information on the Society and it's many activities. It has fully interactive membership areas where you can book your place at Society meetings, find exclusive *Sf*AM documentation and much more.

membership options

■ **Full ordinary membership** gives online access to the *Journal of Applied Microbiology*, *Letters in Applied Microbiology* and *Environmental Microbiology*, copies of *Microbiologist*, preferential registration rates at Society meetings and access to the members areas of the website.

■ **Full student membership** confers the same benefits as Full Membership at a specially reduced rate for full time students not in receipt of a taxable salary.

■ **Associate membership** is only open to those with an interest in applied microbiology without it being a prime aspect of their job. For example, school teachers and those taking a career break; on maternity leave, or working temporarily in other areas. It does not provide access to any journals or Society grants and awards.

■ **Honorary membership** of the Society is by election only and this honour is conferred on persons of distinction in the field of applied microbiology. Honorary members have access to our online journals.

■ **Corporate membership** is open to all companies with an interest in microbiology.
Corporate members benefits include:

- Quarter page advertisement in each issue of *Microbiologist* (which can be upgraded to a larger size at discounted rates)
- the opportunity to publish press releases, company news, etc., in each issue of *Microbiologist*
- Full page advertisement in the Members' Handbook.
- FREE banner advert on the Society Website with a direct link to your company site.
- Up to three members of company staff attending Society meetings at members' rate (This means a 50% discount on non member registration rate).

■ **Retirement membership** is available to Full Members once they have retired from their employment and have completed at least 20 years membership of the Society. Retired members are entitled to all the benefits of Full Membership except access to *Journal of Applied Microbiology*, *Letters in Applied Microbiology* and *Environmental Microbiology.*

JOIN US!

You can apply for membership on, or offline. To apply offline, please contact the Membership Co-ordinator, Julie Wright on **01234 326846**, or email **julie@sfam.org.uk.** Alternatively, write to her at:

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president's column

Margaret Patterson reviews the higlights of the Society's 75th Anniversary celebrations

The celebrations to mark the 75th Anniversary of the formation of the Society are now behind us.

Looking back, one of the highlights for me was the summer conference in Edinburgh, where it was a pleasure to welcome back so many of our longstanding members and those who served on Committee over the years.

The year ended with the President's Dinner and this was extra special for a number of reasons. The prestigious venue of the Churchill Room at the Palace of Westminster made an excellent backdrop to the evening, which was attended by 80 people, including past Presidents, Honorary members, several MPs and senior representatives from a variety of biological societies.

The book *The Society for Applied Microbiology — A Short History* written to commemorate the anniversary year, was officially launched at the event and the author, Professor Max Sussman was present to sign copies at the end of the evening. We still have some books to give away to members. If you would like your personal copy, please contact the office as soon as possible – they will be given out on a first-come-first-served basis.

Our after dinner speaker was Sir David King, Chief Scientific Advisor to HM Government. He highlighted the critical role of *Sf*AM and other learned Societies in advising and influencing government policy and strategy. It was good to hear that he shared our view that collaboration with like-minded organisations is as important as individual contribution in ensuring that the voice of applied microbiology is heard. He also said that science communicators and writers are vital, to translate complex scientific principles to scientists in different disciplines as well as to MPs without a scientific background. This, he felt, was one of the many skills of Learned Societies in the UK. Again, this is in line with *Sf*AM's ethos and we actively work with the Science Media Centre in suggesting suitable experts to speak out on applied microbiology topics.

It is clear that applied microbiology issues are continuing to attract strong scientific and public interest. MRSA is still making the headlines and *Clostridium difficile* is catching up as the new microbe for the public to worry about. Our winter meeting, held on the 11th January, took place on the very day that it was announced that government targets to reduce the numbers of MRSA cases by 2008 were unlikely to be met. We had very timely presentations on the role government, hospitals and microbiologists can play in controlling hospital acquired infections, including a update on *C. difficile* and MRSA as well as a range of talks on food borne pathogens and the latest outbreak data and trends (see page 21 for a full report of the meeting). The event was very well attended — around 200 delegates — and feedback from the delegates was very positive. Hopefully, this high attendance will be the trend we see at the other meetings planned for this year. You can find details of our one day Spring meeting on *Broadening Microbiology Horizons* (11th April at Manchester Metropolitan University) and our summer conference on *Microbiology of Fresh Produce* (2-5 July at the Park Plaza Hotel, Cardiff) on pages 24 and 26 or on our new look web site (www.sfam.org.uk). Finally, please remember that if you have been a member of *Sf*AM for more than 1 year, you are eligible to apply to the President's Fund to help towards conference expenses (see page 47).

Dr Margaret Patterson, Sir David King and Mrs Iris Robinson at the Palace of Westminster

Dr Margaret Patterson
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

Welcome to my first column of 2007. I begin this year by telling you that in December last year, unfortunately, Rachel Dowdy the Society's Events Co-ordinator left to return home to Australia.

Before she left Rachel thanked the Society for her time with us and highlighted how much she had enjoyed the role which she had occupied. I am sure all the members who had contact with Rachel would like to wish her all the best in her new life down under (see page 10)!

As our President has highlighted, last year's 75th Anniversary celebrations were rounded off with a very successful President's Dinner which was held at the House of Commons. The after dinner speaker was Sir David King, the government's Chief Scientific Advisor. Thanks are due to Mrs Iris Robinson MP, the Member of Parliament who invited the Society to hold this important event at such a prestigious location. It was certainly a very apt way to round off the 75th year of the Society.

You all should by now have received your membership cards. These will be an annual feature which will be distributed once a member has renewed their membership. We have taken the opportunity of highlighting key Society meeting dates on the back of these cards. Any feedback regarding the cards would be appreciated.

You will shortly be receiving your 2007 Members Handbook. If you require a blue binder to secure the pages of the handbook, please contact Julie@sfam.org.uk and she will send a binder across to you.

The Committee and myself have been actively pursuing alliances with other organisations and Learned Societies which will bring mutual benefits to members of both organisations. For instance we worked closely with the Chartered Institute of Environmental Health in putting part of the recent highly successful Winter meeting together. In addition, we have held discussions with the International Association for Food Protection to explore ways in which we might collaborate. One idea is to offer discounted delegate rates to attend each others conferences, these discussions are ongoing. In addition, we have agreed reciprocal advertising with a number of Societies e.g. Biochemical Society. Here, any appropriate meetings will be advertised in each members magazine. These are just some of the initiatives we are exploring, all designed (amongst other reasons) to raise the awareness and profile of the Society for Applied Microbiology to a wider audience.

Once again in 2007 S*f*AM will exhibit at several international conferences. We will exhibit in North America three times during the year. We will be attending the American Society for Microbiology meeting in Toronto in May. This will be followed with two exhibits in July (IAFP, Orlando, 8th – 10th July and International Food Technology , Chicago, 29th -31st July). In addition to attending these three meetings we will also attend and exhibit at the 14th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms (CHRO2007) to be held in Rotterdam, The Netherlands, 2-5 September 2007. As well as trying to enrol new members we will be very pleased to meet all our members at these events. Also, do not forget if you would like to attend these meetings and you have insufficient funding why not apply for one of Society's grants e.g., President's Fund? The main criterion (other conditions do apply) for this award is that you must have made at least two subscription payments (further details on page 47). So if you are planning to attend please stop by the stand and collect your members' lapel badge.

As I briefly mentioned earlier, the recent Winter meeting covering Food and Health was a resounding success with over 170 delegates. Thank you to all our speakers for making it such a first rate meeting (see page 20 for a full report). Once again in this issue please see details of the Spring one day meeting and Summer Conference, these can be found on pages 24 and 26 respectively.

Philp Wheat
Chief Executive Officer

membership matters

Stay in **touch!**

We strive to keep in touch all our members and hopefully you will have been receiving your monthly e-bulletin to your email address. The aim is to keep you up to date with S*f*AM events and members news. To ensure that you are kept up to date, if you are not receiving these bulletins then please contact membership co-ordinator, Julie Wright on +44(0)1234 326661 or email her at **julie@sfam.org.uk** and let her know your up to date email address. Also, if you have any news that you think our membership should be aware of, then please send it to Communications Officer, Lucy Harper **(lucy@sfam.org.uk).**

History Book

Have you requested your copy of the History of the Society book? We still have a few remaining to any members who wish to get hold of a copy. All you have to do is write to communications @sfam.org.uk or phone us on **+44(0)1234 326661** and we will send a copy to you. All postage and packaging is free of charge so contact us soon to be in with a chance of getting hold of a copy.

Sponsor a new Member of the Society and win a £50 Book Token!

If you feel you could be our next winner for 2007, and would like some promotional material to help you recruit new members please contact
Julie Wright, Membership Co-ordinator on 01234 326661 or email julie@sfam.org.uk.

Goodbye

It is with regret that we announce that two of the S*f*AM team have, for different reasons, left their association with the Society. **Professor Nigel Poole OBE** will keep in touch with the Society through his role as Chair of the European Liaison Group of the Biosciences Federation, but his work as our Public Affairs Executive ended at the end of 2006. We would like to thank him sincerely for all the great work he has done in helping to raise the profile of S*f*AM.

The second 'goodbye' goes to **Rachel Dowdy**, our Events Organiser. At the end of 2006 she waved goodbye to head for the warmer climes of her homeland, Australia. I'm sure all who met Rachel at our meetings in 2006 will wish her well for the future and know that she will be sorely missed.

Thanks go to Rachel for making the Society a bright and happy place to work!

Chief Executive of Biosciences Federation **receives OBE**

S*f*AM would like to congratulate **Richard Dyer**, CEO of the Biosciences Federation (BSF) on the receipt of an OBE in the Queen's New Year Honours List. Richard receives the award for services to biology, particularly his contribution to the Babraham Institute and Babraham Research Campus.

Calendar Erratum

Our sincere apologies go to **Henriette M C Put** who kindly contributed a number of photographs to the S*f*AM calendar. Her name has been incorrectly spelt 'Putt' instead of the correct spelling 'Put.'

New **Members**

We would like to warmly welcome the following **new members** and hope that you will participate fully in the activities of the Society.

Australia

I M Al-Bulshi

Egypt

A Y G Hesham

Nigeria

O Bello

Uganda

F J Hawumba

United Kingdom

P C Barbedo; K L Blackett; S H Cabry; Li Chin; S A Chisholm; J Collins; R Comey; T Gardner; K Gkatzionis; S Goddard; S W Gould; D Harrington; S Hiom; C E Jennings; G L Kay; S Kelleher; N J Lakdawala; S Leakey; S Mande; L Manickan; C K Mason; J McQuillan; C Micallef; A Okechuku; T C Okoye; R K Poole; B L Price; K J Roberts; L Sonola-Omitowoju; L Stevenson; J Stewart; L Stone; A Summers; R Taylor; J Thompson; A R Timms; J Wright

USA

M B Cole; K Duddleston; P Schloss

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Bio-Stat Healthcare Group
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Call for S*f*AM Journals

Digitisation of S*f*AM Journal Archive

Blackwell publishing, the publishers of the Society's journals, are in the process of digitising the Society Journals for an archive, which, when complete will be **accessible free of charge to all S*f*AM members.** However, there are a number of volumes missing from their collection. To make the archive complete, Blackwells are looking for hard copies of Vol.1–13 of the then **Proceedings of the Society for Applied Bacteriology.** We are assured that all copies will be returned to their owners after use. So if you, or a friend are in possession of any of these journals, please contact Vicky Johnson, Journal Publishing Manager, Blackwell publishing at: vicky.johnson@oxon.blackwellpublishing.com **Tel: +44(0)1865 476244**

Call for nominations for W H Pierce Prize Award

Do you know a young microbiologist (under 40 years of age) who has made a substantial contribution to microbiology? If so, why not nominate them for this prestigious and substantial award which is worth £2,000. The award was instituted in 1984 by Oxoid to commemorate the life and works of the late W H (Bill) pierce, former chief bacteriologist at Oxoid Ltd and a long-time member of the Society. The prize is presented annually at the summer conference. Full Members wishing to make a nomination for the 2007 prize should write in confidence to the Hon General Secretary, Dr Anthony Hilton, at the Society Office in Bedford, including a full cv of the person nominated and a letter of support. Please note there are no official forms for this award.

Closing date for nominations is 27 April 2007.

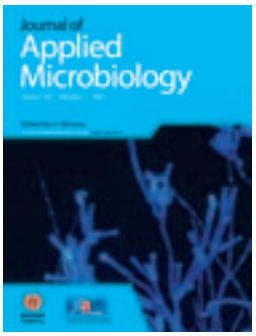
Please note that application is through nomination by Full Members of S*f*AM only.

Call for Nominations to Committee

Four members of committee are due to retire in July 2007 after their three years of service: Dr John Coote, Professor Geoff Hanlon, Dr Karen Stanley and Dr Susannah Walsh; thus there will be four vacancies to fill in July 2007. Nominations are invited from all full members of the Society for these vacancies. Nominations must be made in writing and received by the Society Office by 9 May 2007. Should nominations exceed vacancies, election will be by a system of postal voting arranged by committee.

President's Fund

The President's Fund provides limited grants to ALL S*f*AM members to assist them to attend scientific meetings or workshops related to their area of work. Awards are made at the sole discretion of the Honorary President. Please note that this Fund is open to members of all ages! For more information see page 47 or visit the website.



Top 5 most downloaded articles for *Journal of Applied Microbiology:*

1) Novel antiviral agents: a medicinal plant perspective. S.A.A. Jassim, M.A. Naji
Volume **95**, Issue 3

2) Antimicrobial agents from plants: antibacterial activity of plant volatile oils
H. J. D. Dorman, S. G. Deans. Volume **88**, Issue 2

3) Probiotics and their fermented food products are beneficial for health
S. Parvez, K.A. Malik, S. Ah Kang, H.-Y. Kim
Volume **100**, Issue 6

4) Antimicrobial activity of essential oils and other plant extracts
K. A. Hammer, C. F. Carson and T. V. Riley
Volume **86** Issue 6

5) A history of influenza. C.W. Potter
Volume **91** Issue 4

journal**Watch**

News about the Society's journals

Top 5 most downloaded articles for *Letters in Applied Microbiology:*

1) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity
A. Nostro, M.P. Germanò, V. D'Angelo, A. Marino, M.A. Cannatelli. Volume **30** Issue 5

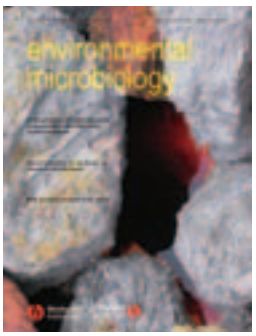
2) *Campylobacter jejuni*
W.J. Snelling, M. Matsuda, J.E. Moore, J.S.G. Dooley. Volume **41**, Issue 4

3) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7
S.A. Burt, R.D. Reinders. Volume **36**, Issue 3

4) Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. S. Satish, K. A. Raveesha, G. R. Janardhana. Volume **28** Issue 2

5) Under the Microscope: *Arcobacter*
W.J. Snelling, M. Matsuda, J.E. Moore, J.S.G. Dooley. Volume **42** Issue 1

Top 5 most downloaded articles from *Environmental Microbiology:*



1) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Veronica Artursson, Roger D. Finlay, Janet K. Jansson
Volume **8**, Issue 1

2) Quorum sensing: the power of cooperation in the world of *Pseudomonas*. Mario Juhas, Leo Eberl, Burkhard Tümmler. Volume **7**, Issue 4

3) Exocellular electron transfer in anaerobic microbial communities
Alfons J. M. Stams, Frank A. M. de Bok, Caroline M. Plugge, Miriam H. A. van Eekert, Jan Dolfing, Gosse Schraa. Volume **8**, Issue 3

4) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Gabriele Berg, Leo Eberl, Anton Hartmann. Volume **7**, Issue 11

5) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. K. N. Timmis, *et al.* Volume **4**, Issue 12

A New Journal for 2008 – ***Microbial Biotechnology.***
Editors: K. N. Timmis (EIC), Juan Luis Ramos, Willem de Vos, Willy Verstraete, Steve Projan and Marty Rosenberg (from 2009)
Microbial Biotechnology has been created to publish papers of original research reporting significant advances (belonging to the upper 25% in the field) in any aspect of microbial applications. It will be a sister of the premier microbiology journals *Molecular Microbiology, Environmental Microbiology* and *Cellular Microbiology*, and joins the extremely successful stable of microbiology journals that also includes the S*f*AM and FEMS journals.

Scope

The Journal will publish original research on microbial applications, including, but not limited to biotechnologies related to green chemistry, primary metabolites, food, beverages and supplements, secondary metabolites and natural products, pharmaceuticals, diagnostics, agriculture, bioenergy, biomining, including oil recovery and processing, bioremediation, biopolymers, biomaterials, bionanotechnology, biosurfactants and bioemulsifiers, compatible solutes and bioprotectants, biosensors, monitoring systems, quantitative microbial risk assessment, technology development, protein engineering, functional genomics, metabolic design, systems analysis, modelling, process engineering and biologically-based analytical methods.

■ Journal Structure ■ Research Articles
■ Brief Reports ■ Rapid Communications
■ Reviews ■ Minireviews ■ Genomics Update
■ Netwatch ■ Editorials ■ Correspondence

In addition, other items relating to the Journal content and biotechnological applicability will be regularly featured.

Special Issues

Each year, one or two issues will be Special Issues devoted to particularly topical subjects, and edited by expert guest editors.

Launch Timeline: Submissions: from April, 2007. First issue: Jan 2008

book reviews

reviewers

The Society receives several new books every week from publishers around the world and we are always looking for enthusiastic additional reviewers who have an interest in the subjects covered.

There is an up-to-date list of titles available for review at the Society Office.

To make an offer to review any book simply email the Editor of *Microbiologist* at: lucy@sfam.org.uk. In return for your efforts you get to keep the book!

Titles for review and book reviews published in *Microbiologist* will soon be available on the website.

Differential Display

Ronald A. Leslie and Harold A. Robertson
Oxford University Press, UK, 2000
ISBN 0-19-963759-8.
£38.50
xviii + 255 pages
Reviewed by Russ Grant

This book is old by current molecular standards (2000) but deserves a review since its main topic is still relevant. Interestingly (and with the benefit of hindsight) are the comments regarding the potential use of differential display against the developing techniques (now developed one could argue) of microarray – using the tortoise and the hare story characters as a metaphor for differential display and microarrays!

As a brief reminder, differential display involves in essence the application of reverse transcriptase polymerase chain reaction (RT-PCR) to produce a 'fingerprint' of expressed genes that can be compared between libraries. Other methods deriving from this include (from the date of the book and not accounting for further progression) subtractive hybridisation, serial analysis of gene expression (SAGE) and of course, microarray technologies; all are included here.

The first few chapters (all of which are in a journal paper/book hybrid style) deal with some of the more general aspects involved in the 'process' of differential display, and some 'recent' (again note the date) advances, before moving on to some very useful practical tips. The rest of the chapters deal with more individualistic specific areas including arbitrary primers and antisense approaches as well as other modifications for the methodology and technique. Uses of RT-PCR in single cell organism and plant tissues are also featured.

Throughout the book there are good examples of actual experiments showing successful use, with detailed protocols providing a detailed walk-through that can be followed. Problems encountered and experienced are mentioned, and these will be of use to those doing such procedures. This also provides one of the slight downsides to the book since many of the methods are repeated in each chapter. In the way in which the chapters are presented this cannot be avoided and since a reader is unlikely to read every chapter it will not really prove annoying. Other repetitions occur in the introductions, with many explaining the same principles.

If there are any other drawbacks then it is perhaps the books very medical nature, with little mention of any of the numerous other scientific areas that differential display can be used in.

In conclusion this book is a useful resource, but rapid developments in the field, especially in microarray that has now become microchip based with sequences on allowing mass 'displaying' mean it is not as good as its more up-to-date counterparts. For £38.50 this was good when released, but not now in such a fast moving field with current journal papers available on-line with better know-how.

Cellular Microbiology

Pascale Cossart, Patrice Boquet, Staffan Normark and Rino Rappuoli
ASM Press, Washington DC, 2005
ISBN 1-55581-302-X
Reviewed by Sarah Butler

Cellular Microbiology was first published in 2000, giving both students and scientists a valuable source of reference in an emerging field of microbiology. However, this relatively new field has gone through a dramatic revolution requiring this second edition which incorporates genomic and proteomic studies of the microbial world.

The book is divided into 23 chapters, introducing five new chapters and updates existing ones. The first three chapters provide the reader with an overview of microbial pathogens, bacterial human pathogen genomes and cell biology. These are an excellent start for those who have limited knowledge of the subject or need to refresh their memories on certain areas. Chapter two provides detailed information on many significant human pathogens including the well known *Escherichia coli* and *Salmonella* spp.

The next few chapters provide detail on host cell surfaces including sites of pathogen interaction, bacterial adherence and signalling, bacterial toxins, secretion systems in both animal and plant interacting bacteria and interaction of pathogens with the immune system. Chapter eight provides a significant amount of detail on plasma membranes, their structure including lipids and their organization.

Chapters 19 to 21 are particularly useful in the practical approach to cellular biology including a whole chapter of information on electron microscopy providing both students and scientists handy tips on its use. DNA arrays and proteomics are discussed in chapter 21 allowing the reader to understand not only what they are, but also the design and production of arrays as well as a short but useful section on the applications of these highly impacting new technologies developed within the last five years.

The penultimate chapter is dedicated to the cell biology of virus infection which is at the foremost of technology and a new addition to the book. This gives the reader the ability to increase their understanding of the interactions of viruses with cells which will in turn allow the development of new strategies for combating viral infections.

There are many useful illustrations, both 'cartoons' and scanning electron micrographs showing the specific binding of Streptococci for example. Just before the beginning of chapter 19 is a section of figures which are fascinating and provide some colour. The majority of the 'cartoon' illustrations are in black and brown which is a slight disappointment to a very well written, organised and illustrated book.

I particularly like the way in which the chapters are written, giving an overview and good background information before heading into in-depth information for the research scientist. At the end of each chapter are useful references that give the reader a basis to obtain further information/ research on a specific topic. The index is clear and was useful for picking out particular areas within the book.

Overall, I have found the book a particularly good place to start when locating information on cellular microbiology and I have found it of particular relevance to my area of research. Personally, I have found it to be more approachable and an easier read than some of the other books in similar fields. It will find its way onto the shelf of many cellular microbiologists!

Cleanroom Microbiology for the Non-Microbiologist (2005)

David M. Carlberg
Boca Raton: CRC Press
pp. 196 + xviii. (2nd Edition)
ISBN 0-8493-1996-X
Reviewed by Max Sussman

The idea of preventing the access of bacteria to sensitive sites, especially pathogenic ones, arose in the mid-19th century from the researches of Joseph Lister. This is how most undergraduate microbiologists would begin to define asepsis. The original application was for the prevention of surgical wound infection and the operating theatre was the 'cleanroom'. The applications of cleanroom technology have now reached into several industries, notably including the manufacture of electronic devices and computer chips.

The concepts of cleanroom design and use are simple and straight-forward and depend on what is second-nature to microbiologists. Many of those who are obliged to use and supervise cleanroom-dependent processes are not microbiologists and for these the practical application of these concepts is more difficult. These difficulties have not been eased by the appearance of international cleanroom standards and new, rapid automated cleanroom monitoring methods. A further complication is the use of cleanrooms in the pharmaceutical industry, particularly in the production of vaccines derived from animal tissues and cultured eukaryotic cells.

Since this little book is intended for non-microbiologists, the first three chapters are concerned with basic aspects of microbiology, and it is not until page 113 that cleanrooms are discussed in detail. Their classification is well-described as are their various applications. The final chapter is devoted to the detection and enumeration of micro-organisms. One aspect that is not adequately considered is that of non-biological particles, which are important in many industries. The illustrations, though apposite, are rather poorly reproduced.

This book will be a useful primer for the non-microbiologist in this important subject.

Quinolone Antimicrobial Agents (2003)

Eds. David C Hooper and Eitan Rubinstein. Washington: ASM Press
pp. 485 + xiv (3rd Edition)
ISBN 1-55581-231-7
Reviewed by Max Sussman

The puzzle of how a double helix in ring form replicates in the real world was solved with the discovery of the topoisomerases, but the quinolones that inhibit them and have become important chemotherapeutic agents were discovered well before their target enzymes. The topoisomerases II (DNA gyrase) and IV act by passing one region of double helix DNA through another and the chemotherapeutic action of the quinolones is by inhibiting this enzymatic action.

The editors have gathered a distinguished group of authors bringing this useful book up to date and it is divided into three parts. The first part considers the basic chemistry and biochemical activity of the quinolones, as well as their structure-activity relationships and the mechanisms of resistance that have been forged against them. An interesting chapter considers the effect of the quinolones on eukaryotic cells and their potential as anti-tumour agents. The second section deals with the pharmacology of the quinolones and their interaction with a number other very important and commonly used drugs. The final largest part of the volume consists of series chapters that discuss the clinical application of the quinolines.

This volume follows the excellent and well-known style of ASM Press. It is edited to a very high standard and deserves to be consulted by those who study or use these important drugs.

BIOSCIENCES FEDERATION

bio **focus**

Richard Dyer has the latest news from the biosciences

The Biosciences Federation is a single authority representing the UK's biological expertise, providing independent opinion to inform public policy and promoting the advancement of the biosciences.

For further information visit:
http://www.bsf.ac.uk/default.htm

Another New Year has arrived. I hope that it brings you all the important things you seek and few of those that you do not.

From a political point of view I fear that the latter will not be true. The discussions about the future of the RAE have now reached a critical point. The momentum towards the abolition of peer review panels and the introduction of a metrics only based RAE continues. This change is supported strongly by many of your employers! External to Government, the main supporters of the metrics only approach are University Vice Chancellors and The Academy of Medical Sciences. All other 'academies', including The Royal Society, The Royal Society of Chemistry, The Institutes of Physics and Biology, and, of course — The Biosciences Federation are strongly opposed to the abolition of peer review panels. We want these panels maintained and we want them informed by robust metrics including those relating to output.

The main argument for change is to reduce costs – including those costs associated with time. We agree that strenuous efforts should be made to implement clear and substantial reductions in the bureaucracy associated with the RAE and believe that there should be serious discussion about how this can be achieved. A metrics only approach will achieve a cost reduction but this is not at all the right route to follow. Why do I write this?

First, because the BSF holds the view that it is potentially dangerous to rely on an algorithm for an activity as critically important as the RAE. We think it essential that there is some wise evaluation of the quality of the data fed into the formula.

Second, because the BSF thinks that a metrics based formulaic approach will disadvantage some areas of the Biosciences. We are particularly concerned about those important disciplines where research is truly excellent but grant income is low and outputs may be relatively sporadic. Research in Systematics is an example where these anxieties are relevant.

Third, and following from the previous point, the BSF thinks it likely that Vice Chancellors will inevitably move to support most those areas of the Biosciences most suited to whatever algorithm that emerges. These areas will, of course, 'do well.'

And finally, and personally, because I have had too many computer generated letters from non-existent Bank managers based on incorrect information or a "mistake". I have always managed to receive an apology and charges reimbursed. I doubt that you will get (m)any apologies out of the RAE!

What can you do about this situation? Well, of course you can continue to support the BSF and I would welcome your ideas about how costs can be reduced effectively and peer review panels maintained. If you do decide to write to me, please make it brief and let me have your views. Some of you could also start an interesting discussion with your employer!

There are quite a few issues emerging that will have an effect on your professional lives. By the time that you read this, I will have met with a Task Force to discuss the BSF response to a paper published by the Research Councils on Peer Review and our response will be on our web site. If you haven't seen the Research Councils' proposals, you might like to download www.rcuk.ac.uk/research/peer/efficiencypr.htm. I don't wish to prejudge our response to these proposals, but I am 100% confident that we will not be 100% supportive — and nor will you!

I am anticipating a busy year. However I don't want all the activity to be reactive. A proactive position, taken at the right time, can often be more influential than "fire fighting". Ideally, I should like some of the issues where we should trigger debate to come from the Member Organisations. If you have thoughts about important matters to address in the next year or so please let us have them via your Council or Committee.

We are quite pleased by the number of 'hits' our jobs link page received during 2006. You may remember that this was a new initiative for us and is aimed to provide a resource for postdocs. I write "quite pleased" because we are fully aware that all web sites can be improved. If you, or junior colleagues not members of the Society, have a thought about how improvements could be made, please contact Dr Emma Southern (esouthern.bsf@physoc.org). In fact, please contact us about any bright ideas that you may have concerning the BSF. I don't promise to pursue them all, but I do promise to "cherry pick" the very best for consideration!

Once again my best wishes for 2007.

Richard Dyer
Chief Executive
Biosciences Federation

Grime Scene Investigation

our policy on the media

We will:

- always do our best to provide facts, information and explanation.
- if speculation is required, explain the rationale behind that speculation.
- desist from hyping a story—whether it is the journalist or the scientist doing the hyping.

On 3 October 2006 the weekly BBC3 TV series Grime Scene Investigation began. Our honorary general secretary, Dr Anthony Hilton starred as co-presenter of the series. Here he tells us what it was like working on a TV entertainment programme along with three of our student members, Jess Rollason, Laura Wheeldon and Tarja Karpanen.

Q Could you set the scene and tell us about Grime Scene — what's it all about?

A Well, Grime Scene Investigation was an eight part series which was commissioned by the BBC with the intention of investigating peoples' homes, looking particularly at the microbes that live with them. The overall philosophy was: how does your lifestyle influence the type of bugs that live with you, on you and around you.

Q So how did you get involved in the programme?

A Well I was contacted by the executive producer at RDF, the production company, who'd been put in touch with me by your good self I believe. He was looking for an applied microbiologist and had used *Sf*AM as a contact base to find someone who was media friendly. So he phoned me and to be honest I was rather cool on the idea at first. I've been involved with filming pilot episodes of programmes before, which have never amounted to anything, and that situation can end up being not only disheartening but a lot of effort and time for no reward. So after a long phonecall, I told him that I didn't really want to be involved. But he phoned me back about a week later to see if I would at least chat to him about the programme. So I met with him, on the set of Scrapheap challenge actually, and it was at that stage that he increased my interest because I realised that the programme had already secured funding. When I look back the opportunity nearly passed me by, but luckily I changed my mind.

Q So, have you always wanted to appear on TV?

A I think part of me is an innate performer. I've always enjoyed playing musical instruments. So as a youngster I was used to and enjoyed the thrill of performing. I suppose giving a lecture is a bit like a stage performance except the words you use are scientific rather than words in a script. Part of me has always been drawn to that kind of, almost exhibitionism, in a way. Most people shy away from it, but I actually enjoy being in the spotlight.

Q Did you find it nerve-wracking at all — how did it make you feel when you were filming?

A I've done quite a lot of TV news interviews in the past and that's been my only other TV experience really. So when we first started filming for Grime Scene I had to make the transition mentally from news interviews which are often very formal to working with a comedian in a non-scripted environment. At first I found it very scary because Rufus (my co-presenter) and I would be in the middle of a dialogue and I'd just dry up and didn't know what to say. I suppose I was thinking I had to get it all exactly right in one take as you would if it was a live interview. One of the turning points for me was when Rufus said that our role as actors or presenters was to provide a varied palate of 'takes' for the editors to make a show out of — it wasn't our job to make the show. As soon as I realised that, I relaxed and got into the spirit of it — a light hearted communication of

media**watch**

microbiology in the news

If you spot a story in the media which you think should feature in this column, please send it to the Editor at: **lucy@sfam.org.uk.**

some important science which was delivered in an entertaining way.

Q I wonder if some scientists might see Grime Scene Investigation as somehow 'dumbing down' science and therefore might not necessarily respect what you've done. What would you say in response to anyone who expressed this opinion?

A I'd say that the programme was designed first of all to entertain but secondly to educate. If you are going to promote the public understanding of science you have to deliver the information in a different way to that in which you'd deliver a lecture to academics. I think the best way to communicate science to the general public is to not necessarily let them know that you're doing it, otherwise they tend to switch-off. I would suggest that the most able communicators are able to identify their audience needs and share the information in a particular way. When you're standing in front of a camera, you simplify things, to get the overall message across. I would hope I have a sufficiently good scientific track record to be given the privilege of simplifying things without my peers thinking badly of me.

Q So… you think what you're doing, as well as being entertainment it's an important educational tool. Do you think it works?

A I do because since the programme has been broadcast we've had a lot of feedback from school teachers wanting copies of the broadcast to show to their classes. I love microbiology, and I'm sure everyone in the Society loves it, but to a non-scientific audience it can be a pretty dry subject. For a lot of people who haven't had the privilege of looking down a microscope or knowing what bacteria are or what they're capable of doing, a certain amount of imagination is required I suppose to believe they even exist. I think that what Grime Scene Investigation did was to visualise these organisms using some pretty specialised pieces of kit — for example the electron microscope. We showed people that they don't live in sterile bubbles - they live with these bugs every day and in the large part they're not adversely affected by them. Hopefully we raised awareness that you can live in harmony with microorganisms and this is important. A lot of peoples appreciation of microbiology is gleaned from the news, where they hear of MRSA, *C. difficile*, *Salmonella*, flesh-eating bugs, anthrax, botulism, bioweapons, and as a result they probably have a negative opinion of microbiology. I think Grime Scene Investigation brought microbiology into peoples' current thinking but not from a negative point of view.

Q If one of your peers did enjoy what you'd done and had been approached by the media to do either a TV appearance or a radio interview, what advice would you give them?

A S*f*AM have referred quite a number of journalists on to me when relevant news stories hit the headlines so I've done a lot of news items with TV and radio interviews. I think the first thing to do in those kinds of situations is to research as well as possible about the subject and try to pre-empt what you think you might be asked. For example if you're being interviewed about a particular microorganism or current event then try to get as much information as you can about the current situation. If you don't prepare then, particularly in a live interview, you can be made to look rather foolish. Knowing some basic techniques about how to deal with questions does help. Live interviews can be stressful and if you can find out what you're going to be asked then that makes them easier. However, a lot of the time you don't know.

For news interviews I would generally recommend that you talk to your Press Officer – most universities and large organisations will have one. There are also other organisations, such as S*f*AM and the Science Media Centre (www.sciencemediacentre.org) who's Press Officers can be called upon to give you good advice about dealing with the news media. If you are asked to speak then it's a good idea to take the opportunity to do so with the support of an experienced Press Officer.

Q How long did it take to do the filming?

A Each episode took three days of filming on location but prior to that we'd have all the culture media preparation which was done here at the University and then afterwards there'd be all the editing and the voice-overs. So in total I would say each episode took three days of filming and six or seven days either side with ancillary bits and bobs.

Q Would you do it again?

A Yes I would I'd absolutely jump at the chance.

Q Finally, a question relating to the series — do you close the toilet before you flush?

A No, not always even though I know that I ought to. I do wash my hands though!

Major Scientific Achievements

There have been several major scientific achievements during the first half of **Med-Vet-Net's** life including:

- the successful submission for a new EU-funded Integrated Project, Biotracer, aimed at improving the traceability of unintended organisms in the food chain
- central repositories for the collection of characterised *Trichinella, Cryptosporidia* and *Giardia* isolates and reagents
- an authorative report for policy makers on 'Towards an integrated approach in supporting microbiological food safety decisions'
- a collection of reports and evaluations on risk assessment in the pre-harvest phase of food-animal production
- published recommendations for future research on Verotoxigenic *Escherichia coli.*
- a unique database of DNA sequences from over 170 European Bat Lyssavirus isolates from eight European countries
- evaluation of the prevalence of *Salmonella* Genomic Island 1 in nearly 400 European isolates of *Salmonella, E. coli* and *Shigella*.
- an international conference on 'Priority setting of foodborne and zoonotic pathogens'
- an algorithm published enabling diagnostic and early treatment procedures in suspected cases of human trichinellosis
- tool development enabling the successful detection of an outbreak of *Salmonella* carrying the 16S RNA methylase gene mediating aminoglycoside resistance.

MED•VET•NET

The half-way mark: **exchanging results, sharing ideas and developing relationships**

It's hard to believe that **Med-Vet-Net** is now half way through its anticipated lifespan of five years! In this issue we will provide an overview of our achievements so far and what our plans are for the future.

Approximately 50% of the resources of **Med-Vet-Net** fund active research projects with the aim of encouraging and enabling scientific integration. Initially the scientific activities of the Network were based around four major thematic topics: epidemiology; detection and control; host-microbe interactions; and risk research. Increasingly, however, this structure has been viewed divisive and more interdisciplinary activities have been developed based on the five-year strategic scientific plan. Nine of the 11 research Workpackages initiated in Year 1 were completed in February 2006. A further 11 research Workpackages were initiated in line with the Strategic Scientific Plan developed in Year 1. To date, an accumulative total of 29 manuscripts are published or in press in peer reviewed journals.

The remaining 50% of funds is for integration activities: administration, project management and communications.

Administration

The Administration Bureau, based at French Food Agency (AFSSA) in Paris, France, are responsible for Workpackage 1 – the administrative and financial aspects of the Network. They have established procedures making network administration more flexible and efficient and have initiated studies on the options for legal status to ensure sustainability of the Network.

Project Management

The Project Management team, based at the Veterinary Laboratories Agency (VLA) in the UK, are responsible for Workpackage 2 – the overall project management of the Network which includes all non-financial planning, monitoring,

quality assurance and reporting. This Workpackage specifically includes scientific co-ordination and strategic planning, development of research skills and expertise, inter-Network scientific communications and the expansion and extension of the Network by external collaboration.

Development of research skills and expertise is an important objective of **Med-Vet-Net**. This capacity building has been achieved through training courses, workshops and short-term missions. Workshops that have been run so far are: (1) *Salmonella* control and (2) Foodborne pathogens and foodborne disease surveillance for new EU member states. Training courses have taken place on: (1) Risk Assessment and (2) Pulsed-field Gel Electrophoresis. These have trained over 40 **Med-Vet-Net** and about 70 external scientists. Fourteen young scientists have undertaken training in other partner laboratories as part of the short-term mission programme. **Med-Vet-Net** has also provided funding for four PhD students.

Two virtual Special Interest Groups focussing on 'New, Emerging and Neglected Zoonoses' and 'Lyssaviruses' have been established to build a critical mass of expertise from within and outside the Network. The Annual Scientific Meeting has also become a very successful event, with the last meeting, held during May in Malta, attracting over 190 delegates including, for the first time, 42 external scientists. There were 70 short scientific presentations made by delegates, and 150 poster presentations. Five keynote speakers from North America, Israel and Europe were invited and a workshop was held on 'Networking for Food Safety' with contributors from Canada, Denmark and the United Kingdom.

Expanding **Med-Vet-Net** activities is also a strategic goal and efforts continue to raise awareness of Network activities with other zoonoses-related groups from around the world. The transatlantic network on food safety EUUS-SAFEFOOD has now been running for a year, and is providing opportunities for joint meetings with the North American network FSRRN and short-term missions in the USA. A further collaboration with the US Food Safety Research Consortium (FSRC) has resulted in a joint international meeting on 'Priority setting of foodborne and zoonotic pathogens' in Berlin in July 2006.

Communications

The Communications Unit, based at the Society for Applied Microbiology (*Sf*AM) in Bedford, UK, is responsible for Workpackage 3 – the Spreading of Excellence both within and outside the Network. The Communications Team has built a public and private website. The private website has been extensively developed to allow greater online collaboration via workplace areas which allow the confidential exchange and discussion between scientists. Online web-conferencing with web cameras has also been introduced to allow scientists to meet online when face-to-face meetings are not possible. The first Science Communication Internship was completed and the next programme, now consisting of four modules is underway with five participants. The Unit also continues to produce the monthly newsletter, *MVN News*, which is now distributed electronically as an e-bulletin, and work is underway on a stakeholder magazine. Various articles like this one continue to be published in magazine, trade journals and online. Overall the success of the communications strategy means that **Med-Vet-Net** activities are now recognized worldwide.

The future

It is evident that **Med-Vet-Net** is progressing extremely well. The integration and co-ordination of research on zoonoses within Europe is working effectively. Scientists are exchanging results, sharing ideas and developing relationships. The upcoming integrated work-programme focuses on the identified needs for research, and has implications for international research and statutory regulations. The next two and a half years will see **Med-Vet-Net** focus on consolidating the current research programme and also developing resources to specifically address identified research gaps in host-pathogen interaction and the accuracy of disease surveillance. New initiatives include work that will support:

- policy decisions such as priority setting and risk assessment
- skills and resources development in neglected areas such as virology and Containment Level 3
- maintenance of research reference resources such as repositories for some zoonotic parasites
- evaluation of the prevalence of newly identified food pathogen issues such as novel antimicrobial resistances
- application of new technologies such as DNA arrays and spatial epidemiology to major food-borne pathogens.

This article is based on the Executive Summary of the **Med-Vet-Net** Annual Report 2006.

If you would like a printed copy of the Report please contact Teresa Belcher on:
+44 (0)1234 271020,
or email: teresa@sfam.org.uk

med-vet-net

Med-Vet-Net is a European Network of Excellence that aims to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food science research. Comprising 16 European partners and over 300 scientists, **Med-Vet-Net** will enable these scientists to share and enhance their knowledge and skills, and develop collaborative research projects. **Med-Vet-Net** officially commenced on 1 September 2004, and is funded to the value of €14.4 million for five years.

information

For more information about **Met-Vet-Net**, visit:
http://www.medvetnet.org/ or contact Teresa Belcher on:
+44 (0)1234 271020

Winter Meeting 2007 Report

Food and Health

information

For more information about the Society's meetings please visit the website at: www.sfam.org.uk

You can also find details of this years' Spring Meeting and Summer Conference on pages 24 and 26 respectively of this issue of *Microbiologist*

This years' Winter Meeting was held at the same venue as last years', the elegant Royal Society, Carlton House Terrace, on 11 January 2007. The meeting was introduced by President Dr Margaret Patterson who also chaired the plenary session including the Denver Russell Memorial Lecture: *Naturally Occurring Micro-organisms and their Resistance to Physical and Chemical Agents.*

The AD Russell Memorial Lecture was instituted in 2005 to commemorate the life and works of the late A. Denver Russell (1936-2004). This second memorial lecture held at the Royal Society, London, was originally scheduled to be given by Martin S. Favero, PhD, Director, Scientific and Clinical Affairs Advanced Sterilization Products, Johnson & Johnson. However, due to unforeseen circumstances, Dr Favero was unable to attend the meeting. The lecture was very kindly given by Mr Charles Roberts (Director of R&D, Biocides, Advanced Sterilization Products, Johnson & Johnson, USA). The key issue addressed in this lecture was the observation that naturally occurring microorganisms, for example, *Pseudomonas aeruginosa* and non-tuberculous mycobacteria for example *M. chelonae* and *M. fortuitum* are more resistant to chemical germicides and physical stresses such as heat compared strains cultured on conventional laboratory media. Mr Roberts focused on this fascinating concept further by describing data showing how naturally occurring *P. aeruginosa* was able to reach high concentrations of approximately 10^7-10^8 cells/mL in distilled water kept at 25°C in about 72 hrs, compared with subcultured cells of the same microorganism which reached extinction in the same period. He went on to comment how these findings have far reaching impact in several fields, for example in the clinical setting where *P. aeruginosa* has been shown to grow to high levels in haemodialysis fluids and in biofilms on haemodialysis components thus giving rise to the fevers and rigors often considered to be a 'normal' reaction to the dialysis process. These important findings consequently led to the development of guidelines for the prevention of bloodstream infection and pyrogenic reaction associated with haemodialysis.

In addition, Mr Roberts went on to describe how naturally occurring bacterial spores in soil also have far greater resistance to dry heat sterilisation than pure cultures of known sporeformers and pure cultures of resistant spore isolates from soil. Interestingly, this concept has been applied for controlling sterilisation of a broad spectrum of environments, not least some of the spacecraft on NASA's Mars Exploration Program. Both the Viking (1969-75) and Rover (2003-2011) spacecraft were sterilised using naturally occurring spores to control the process.

In these days where the word 'superbug' frequently appears in the press relating to hospital infection, Mr Roberts concluded

his fascinating lecture by informing S*f*AM delegates of the new 'superspore': a spore from a new species of *Bacillus*, *B. xerothermodurans*, *sp.nov* which demonstrates extreme resistance to dry heat. Indeed, these spores have phenomenal D-values of D_{125} (139 hours) and D_{135} (24 hours). However, whilst this extreme resistance to dry heat adds to the ever-increasing list of resistance properties being detected in microorganisms, interestingly, these spores appear to be fully sensitive to wet heat sterilisation. The practical applications of these properties are currently under investigation.

Following the superb A D Russell Memorial lecture S*f*AM delegates were privileged to have a presentation by Professor Brian Duerden (Department of Health, UK) entitled *'What role can the government play in controlling hospital acquired infection?* On a day when MRSA was again taking centre stage in the press, this was a very timely lecture addressing a question which was in no doubt in many of the delegates' minds. Professor Duerden opened his talk with current infection rates associated with MRSA bacteraemia and *Clostridium difficile* and emphasized that a combination of biology, politics and performance management is needed to address the problem of healthcare associated infection (HCAI). In an era where microbiology (new antibiotics, new diseases), modern medicine (increased life expectancy, complex surgery, cancer treatment) and biological factors (microbial populations, human populations and behavior) all contribute to HCAI it is essential to know the size of the problem before it can be addressed.

The first change of direction by the government came in 2001 with the implementation of mandatory surveillance of MRSA bacteraemias within healthcare. Professor Deurdon went on to describe how this was soon followed by a string of government publications to deal with the issue of HCAI; all aimed at halving the number of MRSA bacteraemias by 2008 : *'Getting Ahead of the Curve'* (2002); *National Audit Report* (2004) *'Winning Ways'* (2003); *'Towards Cleaner Hospitals and Lower Infection Rates'* (2004). Professor Deurden explained that this brought in the performance management structures of the DoH and NHS to hold a Trust's management accountable for reducing HCAI.

In order to help individual Trust's achieve their target the DoH developed a series of tools to help improve infection control activities for example 'Saving Lives' which focused on 'self-assessment' and Care Bundles (asepsis, catheter care, surgical site management, urinary catheters, ventilator management). In 2006, The Health Act (2006) made infection prevention and control a legal obligation on all NHS bodies. Professor Deurden concluded by stating that the government's role in controlling HCAI is through measurement, performance management, professional support and legislation. The overall goal of the government is to change human behavior to overcome the biological problem of HCAI and to change the mindset in the NHS by making the environment safe place *before* delivering its specialist care.

The morning plenary session concluded with a splendid lecture from Dr Bob Adak (CDSC, HPA, UK) who addressed the global problem of food poisoning and the risk of specific foods. Dr Adak commenced his presentation by discussing the complexities in obtaining reliable data for surveillance of indigenous foodborne disease (IFD) and then presented data from the infectious intestinal diseases (IID) study whereby estimates of the pathogen-attributable burden of IFD were made. Dr Adak continued by describing how the IID study formed the basis on which further methods for estimating the burden of illness and risk for specific foods (e.g. poultry, eggs, red meat, seafood, fruit and vegetables etc.) were

developed. By using mathematical modeling, surveillance data from England and Wales (E/W) could be used to produce food specific estimates for IFD for primary care, hospitalizations, hospital occupancy and deaths. Data from the UK National Food Survey were used to estimate consumption rates by food and food-specific risk was calculated as the number of illnesses per million servings.

The study demonstrated that the most important source of IFD in E/W is chicken and eggs. These foods are commonly eaten and are therefore associated with relatively high risks. Other foods associated with high risk of IFD are

shellfish and turkey but due to the generally low consumption of these foods in E/W pose little IFD risk. Furthermore, beef and dairy produce ranked high in relation to patients being admitted to hospital following consumption but low in terms of risk. Foods associated with a low risk of IFD were salads and in particular, cooked vegetables. Dr Adak concluded his lecture by emphasizing the importance of understanding the difference between the source of IFD and the vehicle of transmission which is essential in implementing effective strategies to reduce foodborne illness.

Tony Worthinghton
Aston University

Session A: Hospital Acquired Infection

Martin Kiernan, Nurse Consultant at Southport and Ormskirk Hospital NHS Trust outlined the changing role of Infection Control teams. In 1990 IC teams were virtually non-existent but now comprise full-time medical microbiologists; a nurse consultant; IC nurses; surveillance nurses and information officers. Increasing government concern over HCAI has led to the production of multiple guidance documents but a recent NAO report indicated that a significant proportion of CEOs still experienced difficulty reconciling IC issues with priority targets. A recent outbreak of *Clostridium difficile* at a major hospital highlighted some of the problems. The local IC team repeatedly expressed concerns over the poor environment, poor practice, lack of isolation facilities and insufficient priority from management. However, senior managers were reluctant to implement advice from either the IC team or the HPA and this led to a second outbreak. Urgent action was only forthcoming when media attention was turned on the hospital. Mr Kiernan then gave examples of how good practice had led to reductions in cases of *C. difficile* indicating that IC teams can make substantial improvements given appropriate support.

Dr Jon Brazier, Head of the Anaerobe Reference Laboratory in Cardiff, gave a talk on the current status of *C. difficile* infections in the UK. Utilising a PCR ribotyping technique developed at Cardiff the predominant strains isolated up to 2003 were characterised. In 2004 an outbreak of a new strain, Type 027, occurred at Stoke Mandeville hospital leading to 150 cases and 12 deaths. This isolate attracted substantial media attention and seems to be associated with increased toxin production and increased morbidity and mortality. Type 027 has since emerged in other hospitals and is now one of three PCR ribotypes that currently cause 75% of infections. Hyper-toxin production was believed to be due to an 18 base pairs deletion in the *TcdC* gene that regulates toxin production but this deletion has since been found in other strains. More work is required to evaluate the true significance of this strain and the epidemiology of *C. difficile* disease in general.

Dr Kevin Towner, Head of Molecular Diagnostics and Typing Unit, Queen's Medical centre, Nottingham described the confusing taxonomic history of the *Acinetobacter* genus before going on to outline its clinical significance. Acinetobacters are common in the environment and are found as commensals on human skin. They constitute a clinical problem because of their ability to persist in the environment; their profound antibiotic resistance (including resistance to carbapenems) and their propensity to cause outbreaks. Three carbapenem-resistant clones are now widespread in Europe and possess carbapenemase enzymes in association with changes in outer membrane permeability and efflux systems. What makes *Acinetobacter* special is that it has evolved molecular mechanisms to capture (and express) resistance genes from other organisms. Sequence analysis of one multi-resistant strain has revealed an 86kb resistance island containing 45 different resistance genes.

Professor Barry Cookson, Director of the Laboratory of Healthcare Associated Infection at the Health Protection Agency attempted to answer the question *"MRSA — do we have the situation under control?"* While there was no straightforward answer to this question it is accepted that the associated morbidity, mortality and socioeconomic burden of MRSA are significant Public Health issues. A number of factors are contributing to the problem of MRSA including increased major surgery on older patients; higher bed occupancy rates and patient transfer between wards. These will influence levels of compliance with infection control and antibiotic stewardship. There was also an issue of education and training for nurses and a need for more pathology training for doctors. The Healthcare Commission's review of infection control arrangements in England are currently being analysed and experiences in Europe can provide important insights and evidence as to how we can best prevent and control MRSA.

> MRSA — do we have the situation under control?

Geoff Hanlon
University of Brighton

Session B: Simmering Issues in Food Safety

The afternoon session entitled "Simmering issues in food safety" developed the theme of Bob Adak's morning lecture on the overall picture regarding foodborne disease in

the UK. Joyce Brown and Paul Cook from the Food Standards Agency (FSA) described how the FSA has tried to meet its initial targets for the reduction of foodborne disease by following a broad based strategy focused on both specific commodity sectors such as reduction of contamination in poultry and on cross cutting areas such as the promotion of HACCP and raising food hygiene awareness in catering and domestic kitchens. For the future however, to target the allocation of resources and align it to risk, the FSA is developing a risk matrix. Built using epidemiological and food intake data, it is intended that this will allow comparison of the various sources of risk from food and ultimately comparison of the marginal cost of risk reduction options. A successful and robust matrix is likely to play an important role in determining the future activities of the FSA and ensure the most cost effective interventions to reduce the overall risk of foodborne disease.

One of the "Four Cs" in the FSA's food hygiene campaign was cross contamination during food processing and preparation. Frieda Jorgensen from the University of Bristol reviewed a number of studies on the persistence and transfer of *Campylobacter* and *Salmonella* in food preparation environments. While *Campylobacter* cannot grow in food preparation environments, is less resistant to environmental stresses and generally persists for shorter periods than *Salmonella*, the higher incidence of *Campylobacter* on poultry, the relatively low infectious dose and the kinetics of survival still indicate that cross contamination is an important risk factor in the high number of cases caused by *Campylobacter*.

Another group of organisms that are unable to grow in the food preparation environment yet are important causes of foodborne illness are the enteric viruses, particularly norovirus and Hepatitis A. Mike Carter from the University of Surrey described how these viruses, unlike rotavirus, are mainly associated with infections in adults and how the consequences of hepatitis A infection become more severe with increasing age at infection. One particular difficulty with these viruses is distinguishing between cases acquired from food and those from person to person contact. This was well illustrated by the different estimates of the proportion of foodborne cases arising from national studies in the UK (10%), Australia (25%) and the United States (43%).

Foodborne viruses pose particular problems associated with their detection and this is one reason why they are an unsuitable subject of microbiological criteria for foods. This is not the case for bacterial pathogens however and a new European Commission Regulation on Microbiological Criteria for Foodstuffs came into force on 1 January 2006. Linden Jack of the FSA and a participant in many of the negotiations on this regulation outlined the scope and intended operation of the regulation which has been viewed with some apprehension by the food industry. She emphasised how it is intended to fit in with established food safety management procedures and reassured the audience that it should not lead to the wasteful diversion of resources to increased product testing.

The last speaker of the afternoon was Jim McLauchlin of the HPA's Food Safety Microbiology Laboratory who discussed emerging and re-emerging problems in microbiological food safety. Taking a quotation from Hans Zinsser's 1935 book entitled "Rats lice and history" that "nothing in the world of living things is permanent or fixed" as his text, he described the various social, economic and biological factors that contribute to the changing pattern of foodborne disease. To illustrate this, he covered a number of different topics ranging from the changes in micro-organisms themselves, such as the evolution of VTEC and multiple antibiotic resistant salmonellas, to the effect that East European immigration into the UK has had on botulism statistics over the last few years.

Martin Adams
University of Surrey

Spring meeting 2007

Broadening Microbiology Horizons

Manchester Metropolitan University

Wednesday 11 April 2007

CPD ACCREDITATION

Seven credits have been awarded for this meeting

Programme

09.30–10.30 **Arrival / Coffee / Registration / Trade Exhibition**

10.30–10.35 **Chairman's Welcome**

10.35–11.00 **"Lumping and Splitting" – latest developments in typing methods**
Andrew Fox, Health Protection Agency, Manchester

11.05–11.35 **Latest Developments in Detecting Yeasts and Fungi**
David Denning, South Manchester University Hospital NHS Trust

11.35–12.05 **Use of Bacteriophages as Treatments**
Geoff Hanlon
University of Brighton

12.05–12.35 **An update on Microbiocides**
Jean Yves Maillard
Cardiff University

12.35–14.00 **Lunch / Trade Exhibition**

14.00–14.30 **Dental Microbiology**
Peter Gilbert, University of Manchester

14.30–15.00 **Use of Silver in controlling wound infections**
Val Edwards-Jones, Manchester Metropolitan Manchester

15.00–15.30 **An Update on Rabies**
Tony Fooks, Veterinary Laboratories Agency

15.30–16.00 **Near Patient Testing**
Andrew Sails, Health Protection Agency, Newcastle

16.00 **Meeting Closes**

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

BOOKING FORM and INVOICE

SfAM SPRING MEETING WEDNESDAY 11 APRIL 2007

Broadening Microbiology Horizons

Manchester Metropolitan University, Wednesday 11 April 2007

Only ONE person per form please. If additional forms are required please photocopy this one. CLOSING DATE FOR REGISTRATIONS: Friday 16 March 2007. A LATE BOOKING FEE of £30.00 will be applied to all bookings made after this date.

Cancellation Policy: Up to 30 days prior to the event all cancellations will be subject to a 10% cancellation fee, up to 14 days prior to the event there will be a 50% cancellation fee, and no refunds will be given within 7 days of the event.

DELEGATE FEES

Whole Conference Rate: inc. of registration fee, coffee breaks and lunch. **Please tick the applicable box for the fees you are paying:**	**SfAM Full Member**	**SfAM Student Member**	**Non - Member**	**IBMS Member**
	£50.00 ☐	£25.00 ☐	£100.00 ☐	£60.00 ☐

If you are a non member and wish to attend this meeting we will give you **£10 off** your 2007 membership fee

YOUR COSTS

Charges - please tick the applicable box(es)	Amount
☐ **Whole Conference Rate:**	£
☐ **LATE BOOKING FEE** Payable for all bookings made after Friday 16 March 2007	**£30.00**
TOTAL AMOUNT REMITTED:	£

YOUR DETAILS

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Organisation/Affiliation:_____

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_____ **Postcode:**_____

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Please indicate any special dietary or other requirements (such as disabled access):_____

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● **For all participants: The Society DOES NOT INVOICE for conference fees. Please treat your completed booking form as an invoice. Cheques must be in £ STERLING ONLY and made payable to 'The Society for Applied Microbiology'. Foreign cheques/ drafts MUST be negotiable for the full amount due. Please note that AMERICAN EXPRESS and DINERS CARDS are NOT ACCEPTED.** However the following credit and debit cards are acceptable: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

☐ **Cheque enclosed** ☐ **Please charge my *Mastercard/Visa card /Debit card*** *(please delete inapplicable items)*

TOTAL Amount enclosed/ to be debited: (*Remember to include your LATE BOOKING FEE if booking after 16 March 2007) **£** _____

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Tel: 01234 328330. Fax: 01234 328330. Email: meetings@sfam.org.uk

Summer conference 2007

Including the Lewis B Perry Memorial Lecture - ***'Bacterial anti-cancer vaccines: a science frozen in time'*** given by Dr Peter Green, NCIMB, Aberdeen

Microbiology of Fresh Produce

Park Plaza Hotel, Cardiff, UK
Monday 2 to Thursday 5 July 2007

Including sessions on:

- Organisms and the plant
- Public health aspects of fresh produce
- Intervention strategies
- The industrial perspective

CPD ACCREDITATION applied for

For the latest programme please visit us online at **www.sfam.org.uk**

call for posters

There will be an opportunity during the meeting to present posters in any relevant subject area. Abstracts of less than 500 words, to include aims and objectives, brief methodology, results, conclusions and implications of the work, should be submitted only as a Microsoft™ Word document attachment to an email addressed to julie@sfam.org.uk with the subject line 'Summer Conference 2007 submission'. Closing date for submissions is 11 May 2007.

Programme

Monday 2nd July

14.00 onwards - Arrive and Register

18.00-18.50 **Lewis B Perry Memorial Lecture (National Museum of Wales): 'Bacterial anti-cancer vaccines: a science frozen in time."**
Peter Green, NCIMB, Aberdeen, UK

19.00-20.00 **Drinks Reception – National Museum of Wales.**

20.00 **Evening at leisure**

21.30 **Quiz Night**

Tuesday 3rd July

09.00-09.35 **The problems with fresh produce: an overview**
Mike Doyle, University of Georgia, USA.

Session 1. Organisms and the plant

09.35-10.10 **Zoonotic pathogen interaction with the microflora of the growing crop phylloplane**
Bill Keevil, University of Southampton, UK

10.10-10.45. **Microflora of plant parts intended for consumption**
Jo Heaton, University of Lancaster, UK

10.45-11.15 **Coffee/posters**

11.15-11.50 **Non-culture based approaches to examining the microflora of salad vegetables**
Chris Dodd, University of Nottingham, UK

11.50-12.25 **Measurement and modelling attachment of bacteria to plant surfaces**
Tim Brocklehurst, IFR Norwich, UK

12.25-13.00 ***Erwinia* soft rots**
Amy Charkowski, University of Wisconsin, USA.

13.00-14.00 Lunch

Session 2.

14.00-14.35 **Fungi quality and safety issues in fresh fruit and vegetables**
Maurice Moss, University of Surrey, UK

14.35-15.10 **Rapid methods to detect quarantine pathogens in imported produce**
John Elphinstone, Central Science Laboratory, York, UK

Public health aspects

15.10-15.45 **Prepared salads and public health**
Chris Little, Health Protection Agency, London, UK

15.45-16.15 **Tea/posters**

16.15-16.50 **Pathogens from organic wastes – incidence and survival**
Michael Hutchison, University of Bristol, UK

16.50-18.00 **Student Session**

17.30-19.30 **Trade Show**

Wednesday 4th July

Session 3

09.10-09.35 **Microbial pathogens – strategies for survival**
Jay Hinton, IFR Norwich, UK

09.35-10.10 ***Burkholderia cepacia* and other opportunistic pathogens**
Speaker TBC
University of Ghent, Belgium

10.10-10.45 **Risk Assessments for fresh fruit and vegetables**
John Bassett, Unilever, Bedford, UK

10.45-11.15 Coffee/posters

Intervention strategies for control

11.15-11.50 **Good agricultural practices**
Robert Gravaini, Cornell University, USA

11.50-12.25 **Novel physical methods for decontaminating**
Stephen James, FRPERC – Langford, UK

12.25-13.30 **Lunch**

Session 4.

13.30-14.30 **Offered papers**

14.30-15.00 **Tea/Posters**

15.00-16.00 **Student presentations**

16.00-16.30 **W H Pierce Prize**

16.30-17.00 **AGM**

19.30-20.00 **Drinks reception, tour followed by Dinner at the Millenium Stadium, Cardiff**

Thursday 5th July

Session 5

09.00-09.35 **Chemical treatments**
Des O'Connor, Microsearch Laboratories, UK

09.35-10.10 **Modified atmosphere storage and packing**
Gail Betts, CCFRA, UK

10.10-10.45 **Microbial transfer in fresh salad processing**
Debra Smith, CCFRA, UK

10.45-11.15 **Coffee/posters**

The industrial perspective

11.15-11.50 **EU microbiological criteria**
Kaarin Goodburn, Chilled Foods Association, UK

11.50-12.25 **Issues with organic produce**
Carlo Leifert, Nafferton Ecological Farming Group, University of Newcastle, UK

12.25-13.00 **Suppliers' Perspective**
David Kennedy, Geest Ltd, UK

13.00-14.00 **Lunch & Close**

BOOKING FORM and INVOICE

SfAM SUMMER CONFERENCE 2 - 5 JULY 2007

Microbiology of Fresh Produce

Only ONE person per form please. If additional forms are required please photocopy this one. CLOSING DATE FOR REGISTRATIONS: Friday 8 June 2007. A LATE BOOKING FEE of £30.00 will be applied to all bookings made after this date.

Cancellation Policy: Up to 30 days prior to the event all cancellations will be subject to a 10% cancellation fee, up to 14 days prior to the event there will be a 50% cancellation fee, and no refunds will be given within 7 days of the event.

FEES

	Full Members	Student, Honorary, Associate & Retired Members	Student Non - Members	Non - Members
Full Conference Rate: inclusive of registration fee, coffee breaks, lunches, Society dinner and accommodation in the Park Plaza hotel for the entire conference	£500.00	£250.00	£500.00	£700.00
No Accommodation Rate:	£195.00	£100.00	£195.00	£350.00
Day Rate: 08.30 - 17.00 hours per day, or part thereof, inc. of registration fee, coffee lunch and tea	£100.00	£50.00	£100.00	£150.00

YOUR COSTS

Charges - please tick the applicable box(es)	Amount
☐ **Full Conference Rate:**	£
☐ **No Accommodation Rate:**	£
☐ **Day Rate** (please tick the DAY(S) you wish to attend): **Tues 3rd:** ☐ **Weds 4th:** ☐ **Thurs 5th:** ☐	£
☐ **I wish to attend the Society Dinner on Wednesday evening (This is included in the Full Conference Rate and the No Accommodation Rate but costs £50.00 for delegates who elect to pay the Day Rate).** Please note that numbers are limited:	£
☐ **Additional overnight accommodation per night inclusive of breakfast: £150.00.** If you wish to stay extra NIGHTS please enter the additional NIGHTS you wish to stay: _____	£
☐ **LATE BOOKING FEE** Payable for all bookings made after Friday 8 June 2007	**£30.00**
TOTAL AMOUNT REMITTED:	£

YOUR DETAILS

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Tel No:_____ **Fax No:**_____ **Email:**_____

Please indicate any special dietary or other requirements (such as disabled access) on a separate sheet of paper

YOUR PAYMENT

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☐ **Cheque enclosed** ☐ **Please charge my *Mastercard/Visa card /Debit card*** *(please delete inapplicable items)*

TOTAL Amount enclosed/ to be debited: (*Remember to include your LATE BOOKING FEE if booking after 8 June 2007) **£** _____

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STUDENTSHIP Application

Only ONE form per student please. If additional forms are required please photocopy this one

The Society meeting you wish to attend

Please tick the meeting you wish to attend: ☐ **SfAM Summer Conference** ☐ **SfAM January Meeting**

About this award

SfAM offers Studentships to enable **student members** to attend Society meetings. These grants cover registration, accommodation, meals (where appropriate) and modest travel expenses. Preference is given to students offering a paper or poster and who have not previously received this award. To be considered for a Studentship grant, please complete this form in **BLOCK CAPITALS** and return it to the Society **no later than 6 weeks before the date of the meeting you wish to attend. Applicants MUST hold / have access to a legitimate UK bank account.**

Your details

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Address:_____

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Tel No:_____ **Fax No:**_____ **Email:**_____

University or College:_____

Your Department:_____ **Position in Department:**_____

Grant authority:_____

Your intended career:_____

Your costs

Expected Travel Costs:_____

Other costs - please specify:_____

_____

Why do you wish to attend this meeting?

Please give your reasons:_____

_____

Your signature:_____ **Date:**_____

(If you need more space for your answer please continue on a separate sheet)

Will you be contributing by offering a Poster or presenting a paper? ☐ **Offering a Poster** ☐ **Presenting a Paper**

Your Supervisor's support

This section **MUST** be completed by your Supervisior or Tutor. Applications which are not supported by your Supervisor will be automatically rejected. **Please give your reasons why the applicant should receive a studentship:**

_____

_____

_____

Supervisor's name:_____ **Tel and extension:**_____

Supervisor's signature:_____ **Position:**_____ **Date:**_____

(If you need more space for your answer please continue on a separate sheet)

In signing this application I agree to reimburse the Society for any costs it may incur in awarding this grant should the applicant fail to attend the conference or fail to notify the Society of their inability to attend the conference within 14 days of the start of the meeting.

Please confirm your agreement by ticking the appropriate box: ☐ **I agree** ☐ **I do not agree**

Please return your completed application by fax or post to: **The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK. Tel: 01234 326661. Fax: 01234 326678. Email: meetings@sfam.org.uk**

Bacteriophage Therapy

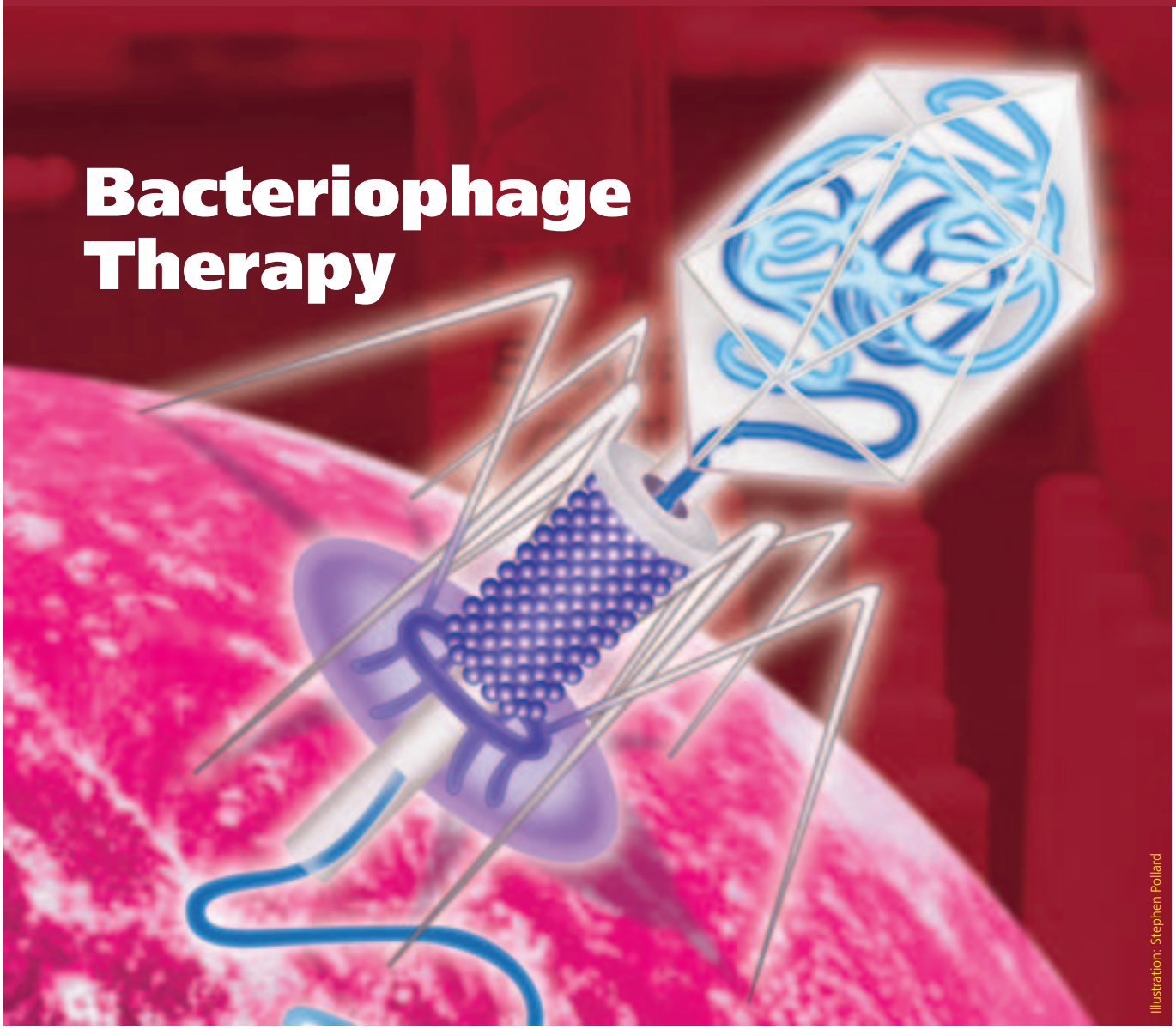


Illustration: Stephen Pollard

Geoff Hanlon discusses antibiotic resistance as a driver for exploring alternative therapies and revisits the potential of bacteriophage therapy

Bacterial resistance to antibiotics is an issue that has been with us almost as long as antibiotics have been available for clinical usage. The phenomenon was predicted by Alexander Fleming in 1945, since he had been aware of it in his laboratory some years earlier, and by 1946 reports showed that 14% of staphylococci isolated from patients in hospitals were resistant to penicillin. The speed with which this resistance then developed would have been a cause for concern since by 1950 the incidence of resistant staphylococci had increased to nearly 60%.

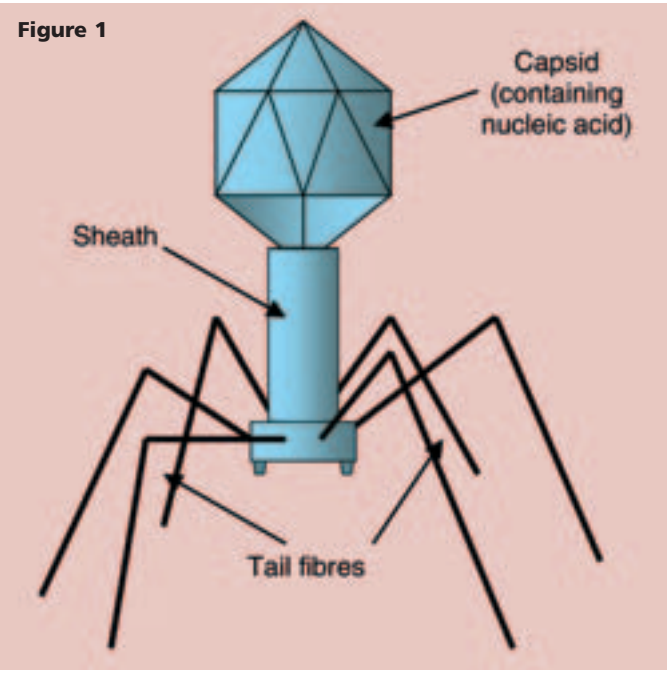
The decades that followed saw the discovery and development of many new major classes of antibiotics to counter the accelerating incidence of antibiotic resistance. Hence the adaptability of the microorganisms was matched by the ingenuity and efforts of the pharmaceutical industry in providing a steady stream of antimicrobial agents with novel mechanisms of action. This led in some instances to complacency and a misconception that the situation was under control. In 1969, the then U.S. Surgeon General William Stewart declared in a message to Congress that with the development of antibiotics and vaccines, infectious diseases had been conquered "..it is time to close the book on infectious diseases, declare the war against pestilence won, and shift national resources to such chronic problems as cancer and heart disease". This declaration was somewhat surprising given that strains of staphylococci resistant to methicillin were produced in the laboratory in 1961 barely one year after it appeared on the market and clinical isolates emerged shortly afterwards.

In the years since then the situation has deteriorated steadily and we are now faced with extreme resistant *Mycobacterium tuberculosis;* vancomycin resistant enterococci; vancomycin resistant *Staphylococcus aureus;* community acquired *Staphylococcus aureus;* carbapenem resistant *Acinetobacter baumanii* and *Ps. aeruginosa* isolates resistant to everything except colistin. Although the public and media attention has been focused primarily on MRSA it has been stated that we are closer to the end of the antibiotic era with Gram negative pathogens such as *Acinetobacter baumanii.*

While the relentless march of antibiotic resistance is, of course, a major concern it is equally worrying that unlike the situation in the 1960s and 1970s the pharmaceutical industry is not now developing a steady stream of new antibiotics to bolster the clinical armamentarium. The Food and Drug Administration in the USA approved 16 new antibacterial

agents between 1983 and 1987. However, the number of approvals for new antibiotics has fallen steadily since that time until during the period 1998 to 2002 only seven were approved while in 2002 there were none. Between January 1998 and December 2003 the FDA approved as many anti-HIV drugs as agents for the treatment of all bacterial infections. It is clear therefore that the pharmaceutical industry is withdrawing from research and development of antibiotics in favour of other pharmacological agents.

Figure 1



A recent survey of the R&D programmes of the 15 largest multi-national pharmaceutical companies revealed that out of a total of 418 agents listed as being under investigation only five (1.6%) were antibacterial agents and none of these represented new classes of antibiotics. It is therefore apparent that given the time taken to bring a new chemical entity from Phase I clinical trials to the marketplace (estimated to be eight years) the prospects of a novel antibiotic being available for clinical use in the next decade are low. The reduction in antibiotic research and development was not a result of falling budgets since the combined R&D expenditures (for 10 of the companies for which data were available) increased 31% over the five year period from 1998 to 2002. The reason for the withdrawal of the pharmaceutical industry from antibiotic R&D despite an illustrious history is not because a lack of ideas on how to tackle the problem but is simple economics. The cost of new drug development has been estimated as between $400 and $800 million per compound. While some drugs can bring in revenue of several billion dollars most antibiotics generate much more modest income, in many cases not even covering their costs. The low return for antibiotics results from their much reduced usage compared to other drugs which a patient may take continuously for the remainder of their lives. In addition the prudent management of potent antibiotics demands that they be kept in reserve for the most serious clinical cases which hardly acts an incentive for further investment.

The consequence is that for a number of infectious diseases the options for effective clinical management are becoming dangerously limited. This has become the driver in the search for alternative strategies to treat infections caused by antibiotic resistant bacterial pathogens. A plethora of non-antibiotic therapies have been investigated recently, many of them natural remedies and often with an extensive history of use, including garlic extracts, green tea, photodynamic therapy, pine cones, tea tree oil, maggots, honey, copper and bacteriophages. Many others have been described (and indeed are available commercially) but are not backed up by rigorous investigation and publication in respected scientific journals. While most of these will not find extensive clinical usage some may eventually be shown to have sufficient merit to act as an adjunct to antibiotic therapy. The remainder of this article will review in detail the potential of bacteriophages in the treatment of bacterial infections.

Bacteriophage biology

Bacteriophages (phages) are bacterial viruses that cannot infect mammalian cells but specifically target bacteria and each phage will only attack one species or in some cases a single strain of bacterium. They are, of course, obligate parasites and while they contain the necessary genetic information to orchestrate their own replication within a host cell they do not have the required elements for generating energy. Most phages exhibit the characteristics shown in Figure 1 whereby the head or capsid is a protein shell usually in the shape of an icosahedron and this contains the viral genome which is most commonly double stranded DNA.

Virulent lytic phages cause lysis and death of the target bacterial cell and these phages are the most usual type used for therapy. Temperate phages integrate their DNA which is replicated when the host cell genome replicates and so daughter cells inherit the viral DNA. These phages have little value in therapy and will not be considered further.

A bacteriophage encounters its bacterial host during random movement and attaches via specific receptor sites present at the tips of the tail fibres to a variety of cell surface components. These may be present on the cell envelope, capsule, flagella or even conjugative pili. The phage genome is injected into the bacterial cell where it is transcribed by host cell RNA polymerase into proteins which redirect the metabolic machinery of the bacterium to manufacture new virus components that are then assembled into complete virions.

Historical perspective of bacteriophages as therapeutic agents

Ernest Hankin is credited with being the first person to observe the potential therapeutic effects of bacteriophage when in 1896 he noted that the waters of the Ganges and Jumna rivers in India possessed antibacterial properties which seemed to reduce the number of cases of cholera in those villages close to the river. He did not realise that the effect was due to a bacterial virus, however, and it was not until 1915 that Frederick Twort in a note to the Lancet made the connection. Two years later Felix d'Herelle claimed to have made similar observations independently from Twort and named the viruses bacteriophages (bacteria eaters). There was considerable acrimony at the time over who should be credited with their discovery although nowadays, in general, they are jointly acknowledged. Whatever the truth regarding d'Herelle's claim of independent discovery there is no doubt

that he was the first person to realise and test the potential of bacteriophages for the treatment of bacterial infections. While working in Paris in 1919 he had in his charge a number of patients suffering from dysentery including a young boy. D'Herelle obtained phage from contaminated stool samples, purified them and tested them both orally and parenterally on himself, his co-workers and even members of his own family. With all the volunteers having suffered no apparent ill effects he gave the boy a single draft of the preparation and he recovered rapidly. Further landmark treatments included four patients with bubonic plague in 1925. Antiplague phage was isolated, purified and then injected directly into the inflamed lymph nodes (buboes) of the axilla and groin areas. All patients made a dramatic and complete recovery.

It is perhaps not surprising that these events generated immense interest since they occurred before Alexander Fleming had reported the discovery of penicillin and at a time when bacterial infections were all but untreatable. D'Herelle went on to study the potential of phage therapy in a range of bacterial diseases and successfully treated a large number of patients; a particular success story being the treatment and prophylaxis of cholera in India. His laboratory was also responsible for the production of commercial bacteriophage preparations targeted against gastrointestinal infections, respiratory infections and wound infections. However, as more researchers moved into the field and pharmaceutical companies started manufacturing bacteriophage preparations there were many treatment failures.

With hindsight the failure of phage therapy was almost inevitable and was the result of high expectations on the part of the public fuelling unscrupulous claims on the part of those with commercial interests together with a poor knowledge of phage biology. A scientific argument raged up until the early 1940s as to whether the bacteriophage phenomenon was due to a virus or self-activating lytic enzymes. Even d'Herelle was convinced initially that bacteriophage represented one specie of microbe and so the exquisite specificity of phages was not appreciated nor the difference between lytic and temperate viruses. The preparation and purification of phages at that time was a crude process and often patients were dosed with products containing no viable phage but large amounts of bacterial lysate including endotoxin leading to profound toxic effects. Each of these backward steps had a weakening effect on the case for furthering research into phage therapy. In 1934 a damning report commissioned by the American Medical Association distilled all these doubts and with a growing interest in the emergence of antibiotics phage therapy was all but ignored in Western medicine. It is relevant to have an appreciation of historical failures since it allows us to not only see what went wrong but also to understand the course of events which shaped our current prejudices.

While the development and use of antibiotics flourished in western medicine, the Soviet Union and parts of Eastern Europe persevered with phage therapy driven primarily by economic considerations. The Eliava Institute of Bacteriophage, Microbiology and Virology, which was founded by Giorgi Eliava in close collaboration with Felix d'Herelle in 1923, was the principal research and development centre in the USSR. The Institute still exists today and is located in Tbilisi which is now the capital of the independent Republic of Georgia. The Eliava Institute remains both a major research and development establishment and possesses a wealth of expertise gathered from many decades of clinical usage of bacteriophages. Unfortunately, the majority of the clinical research conducted over this extensive period was not published in western literature and hence has remained obscure. That which has been reviewed is generally considered to be of poor quality. However, poor experimental design does not necessarily mean that the underlying technology is flawed and the sheer weight of evidence demands that we should give phage therapy further consideration. Our knowledge of bacteriophage biology has grown enormously since the early attempts at phage therapy because they have been instrumental in the development of modern molecular genetics. Advances in biotechnological processing and effective clinical trials design means that we now have the necessary tools to properly re-evaluate the potential of phage therapy.

Bacteriophage therapy returns to the West.

During the 1980s a number of western scientists conducted a series of studies to test the efficacy of phage therapy in bacterial infections of animals. This well designed body of work, published in respected western scientific journals showed that bacteriophages were at least as effective in combating bacterial infections as antibiotics and in some cases exhibited significant advantages. This has acted as a catalyst for further research and PubMed lists over 120 papers in 2006 alone under the heading of phage therapy.

In the last few years a number of companies have been formed throughout the world developing new technologies to exploit bacteriophage therapy. In order to obtain patentable intellectual property many of these companies have utilised genetic manipulation of bacteriophages. Examples include the development of phages with altered lysin enzymes, modified adhesion profiles and toxin delivery mechanisms. Other companies are becoming established as phage therapy centres in order to treat patients from the West in countries which are not subject to such strict Regulatory control.

Summary

Bacteriophage therapy promised much in the early 20th century but failed to deliver because the underlying science was not in place to fully exploit the technology. Instead the treatment of bacterial infections in the West over the last 60 years has been dominated by antibiotics. As these begin to fail, perhaps the time has come to revisit phage therapy taking advantage of the substantial clinical experience of scientists within the former Soviet Union and our own much improved knowledge of bacteriophage biology.

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Milton Wainwright tells the story of how, during the early 1900s, consumptives from the Leeds-Bradford area and further afield, claimed to have been cured by breathing in the fumes produced by maggots. Helped by two of his postgraduates, Dr Wainwright has demonstrated that there may be scientific rationale behind these seemingly improbable claims

When maggot fumes cured tuberculosis

Imagine entering hospital to find that living maggots will be applied to your body. This therapy, distasteful to many, is increasingly being used with success on patients suffering from long-standing antibiotic resistant wounds.

The use of such maggot therapy has a long and well documented history. Less well known, is the fact that the gases and volatiles produced by maggots were used to treat pulmonary tuberculosis, a technique we can term, "maggot inhalation therapy." This approach was pioneered not by a medical man, but by a maggot farmer called Arthur Bryant, who reared maggots for fishing bait at his farm in Thornton, near Bradford. Bryant operated what he called a 'Maggotorium', where sufferers of pulmonary tuberculosis could come and breath in maggot fumes in the hope of improving, or even curing, their condition. Standard maggot therapy had long been used to treat tuberculosis of the hip, so it was not an altogether wild idea that, by breathing maggot fumes, pulmonary TB might be cured.

Before beginning maggot farming in the early 1900s, Bryant had himself suffered with tuberculosis and had been denied life insurance as a result. However, after a few months breeding maggots, and breathing the fumes they produced, he was declared fit enough to be given insurance cover worth £200 cover, then a considerable sum. When a little girl was apparently cured by inhaling his maggot fumes, word spread and people flocked to his farm for treatment. In February 1911, the *Bradford Telegraph* organised an open day at the farm when consumptives from all over Yorkshire and further afield came to seek the treatment. For a limited exposure time, they paid sixpence in old money (then the price of admission to a football match); this was then donated to local charities. As a result Bryant was heralded as the Maggot King and his Maggotorium became locally renowned.

How the Maggots were Produced

The maggot farm was sited at Jerusalem Farm at Erlings Quarry, between Denholme and Thornton near Bradford, in what was then the West Riding of Yorkshire. Maggots were grown on waste rendered meat in large tin baths contained in maggot sheds. The meat, which included dead zoo animals, was boiled in an adjacent boiling shed. Every week in the summer Bryant would take four and a half tons of dead meat into four different woods in order to replenish his supply of maggots. From June of each year, he would grow maggots on 15-25 tons of meat, the smell of which could be detected three miles away! The waste from the Maggotorium was sold at low cost to local farmers as a fertilizer, and seems to have been in great demand.

When undergoing inhalation therapy, consumptives would sit in the Maggotorium, besides troughs full of maggots. Here they would inhale the fumes and pass the time reading, chatting or playing card games, and soon become accustomed to the unpleasant smell.

Bryant's Maggotorium

The success of his maggot therapy led Bryant to apply for planning permission to build a sanatorium, where the conditions were to be markedly improved. Denholme District Council passed these plans to increase the size of the facility, but Bryant never got the capital together to realise his new enlarged, and more salubrious, maggot sanatorium. Interestingly, a meat rendering plant still exists at Erlings

Quarry; local residents having recently successfully petitioned against an increase in the movement of trucks carrying foul smelling waste meat to the works.

Bryant published a pamphlet in which he provided a large number of testimonials to the effectiveness of his inhalation maggot therapy. Here are some typical recommendations:

"I have been at the Maggotorium six weeks. I was very bad when I went and could scarcely walk. I have been in another Sanatorium but received no benefit thereby. After six weeks of Mr Bryant's treatment I was able to resume my ordinary duties, and at the present time I am feeling splendid, and can truly testify to the benefit obtained by undergoing the treatment."

"I weighed 8st 9lb when I went to the Maggotorium and after four weeks I weighed 9st. My apetite is much better and I can breathe more freely. My cough is almost gone, except first thing in the morning."

"I have been attending the Maggotorium for two hours every night for four weeks after I had done work. I have been ailing for three years. I think when I have been one more month I shall be alright... I gained six and half pound in the first four weeks."

"I have been to Mr Bryant's Farm after being turned out of Eastby Sanatorium as incurable. I am now working and earning my own living. I am disgusted in the way Dr Kaye has treated this wonderful discovery."

"I have been to the sanatoriums at Ewick and Ventnor Isle of Wight. I was in very bad state when I came to your place could hardly walk, and have been ailing four years. I was pronounced by a Liverpool doctor as cured three weeks ago."

A number of these testimonials refer to requests for 'another box of maggots', or boxes of 'maggot gas', so Bryant seems to have provided a take away service and supplied maggots by post.

The Nature of the Active Ingredient

The pamphlet of Testimonials also provides details of the work of F.W. Richardson, the Bradford City Analyst.

Richardson analysed the maggot gasses and found that they contained: triemethylamine, ammonia, dimethylamine, and traces of skatol and indol; the two amines give maggot gasses a characteristic fishy smell, beneath the predominant smell of ammonia. He commented that a curious feature was the presence of twice as much amine as ammonia. He also noted that trimethylamine was then used in the treatment of pneumonia, cholera and rheumatism. Although he quotes the view of a certain J.V. Shoemaker that ammonia has marked antiseptic value and also acts as a cardiac and respiratory stimulant he was initially (i.e. on 16 May the 1911) dubious that maggot fumes could have a curative effect on tuberculosis. However by 4 July 1911, Richardson had conducted a number of experiments with ammonia and trimethylamine and had found that, even in weak solutions, they can destroy microbes after only a few hours exposure. He then suggested that maggot fumes might indeed have a curative affect and encourages the work to be further continued and the results made known to the public. He concludes:

"If you care to do so, we will not charge you anything for our services as we would freely give them for the furtherance of the commonwealth. I am strongly opposed to any attempt to corner any discovery, the general knowledge of which might be of great service to suffering humanity." Unfortunately, the letter is addressed "Dear Sir" although presumably it was sent to Bryant. Richardson, by the way, was later called upon to investigate the cause of the notorious explosion in an ammunitions factory near Bradford on Monday 21 August 1916.

Confirmation that maggot fumes inhibit *Mycobacterium*

We have recently shown that fumes from the blue blow fly (*Calliphora quadrimaculata*) grown on beef heart prevent the growth of *Mycobacterium phlei.* This is a non-pathogenic *Mycobacterium* that is widely used as a model to avoid using the more dangerous, *M. tuberculosis*, and the cause of pulmonary tuberculosis. Gasses from the *C. quadrimaculata* and the green blow fly (*Phaenicia sercicata*, formally *Lucilia sericata*) grown without meat also produce gasses inhibitory to *M. phlei.* A mixture of the main components of maggot gasses, ammonia, di- and trimethylamine (both used as the hydrochloride) also inhibit *M. phlei*, as does ammonia vapour alone (from ammonium hydroxide). A mixture of the two amines and ammonia (i.e. a simulated maggot gas mix) is also inhibitory to *M. phlei.* It is noteworthy that maggot gasses do not inhibit the growth of *Staphylococcus aureus*, including MRSA, as a result, the inhibition of the growth of *M.phlei* by maggot gases is not a generalised germicidal effect.

In Figure1 the exposed plate is on the right, while the control is on the left; the bacterium also grew anaerobically, and in an atmosphere of carbon dioxide, showing that the inhibitory effect shown was in fact due to maggot gases.

These results confirm the antibacterial activity of maggot gases noted by Richardson, and suggest that there is indeed a scientific rationale behind the claims made by Bryant and his "patients' that maggot gasses improve the condition of consumptives, and are possible curative." Richardson also noted that, according to Dr J.V. Shoemaker, ammonia possesses marked antiseptic virtues and also acts "in a stimulating way on the respiratory centres"; additionally, he refers to the then use of trimethylamine in cases of pneumonia, chorea and rheumatism.

By 1939, Bryant had moved to the village of Finmere in Buckinghamshire, where he continued maggot farming, now advertising himself as the 'Maggot King of Buckingham', although it is not clear if he continued with his maggot inhalation therapy. There is however, reference to another Maggotorium operating in the UK during the Great War; Cope's Cheshire Directory of and Byers Guide of 1914-1915 shows that a slaughterer, by the name of P. Dobbins offered a 'consumptive cure' at his Maggotorium at Saltney in Cheshire.

The fact that millions of people worldwide suffered and

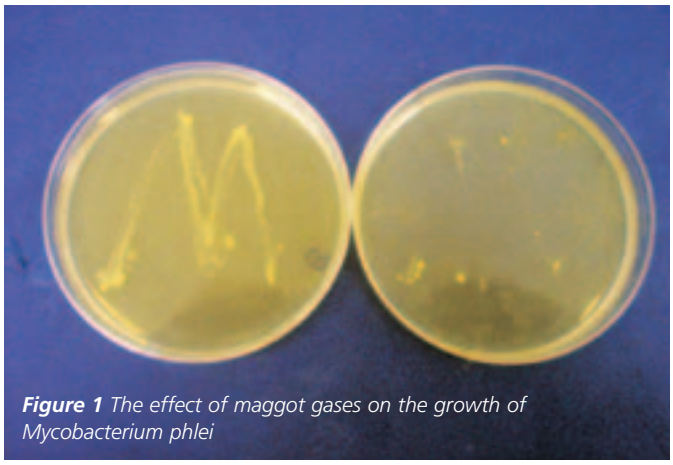

Figure 1 *The effect of maggot gases on the growth of Mycobacterium phlei*

died from pulmonary tuberculosis prior to the advent of antibiotics in the mid 1940s leads to the inevitable assumption that inhalation maggot therapy was not effective in easing the symptoms of TB, or curing the disease. It should be remembered however, that during the 1930s, the British medical authorities were antagonistic to the use of maggot therapy on wounds and burns, despite the fact that this method was widely used with great effect in the US; as a result, it is unlikely that they would have taken kindly to the idea that breathing in maggot fumes could have any beneficial effect on tuberculosis. Maggot inhalation therapy may therefore have been effective, but was shunned by conservative medical administrators and practitioners.

Since there is increasing evidence that the tubercular bacillus will eventually become resistant to all current antibiotics, we may yet have to resort to other therapies; perhaps we have not seen the last of maggot inhalation therapy.

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Microbiology and art: a comfortable combination?

We all know that art provides an opportunity for visualisation and communication, and also that some scientific images are truly beautiful. Links between science and art — or indeed the arts — are diverse, and can be clearly demonstrated when focusing on microbiology.

Over the past few years, I have collected examples of this interdisciplinarity, and produced a lecture which I have given to first year Biology undergraduates at Manchester Metropolitan University (MMU), to audiences at other Universities, conference delegates and the general public. Suggestions for additional examples inevitably arise from the floor, irrespective of the audience, thus aptly illustrating the ability of art to inspire cross-discipline thinking and communication. Some examples (and only some!) are illustrated in this article.

The most obvious link between microbiology and art is perhaps the typically negative microbially-induced spoilage of art and heritage material. Of course, to the deteriogens, the art is but a substrate for colonisation and growth, but to microbiologists and students, this is an important, fascinating (and poorly funded) example of applied microbiology (refs 1,2,3). Indeed several EU funded projects are using microorganisms to assist in the conservation of heritage (BIODAM, BACPOLES, BIOBRUSH). Research publications and presentations on the topic are also substantial (*e.g.*, International Biodeterioration and Biodegradation Society [www.biodeterioration.org] Symposia and Journal; HMS2005, third in a series of International Conferences on the Microbiology and Conservation of Cultural Heritage — the fourth will be in 2008).

More positively, microorganisms themselves have considerable aesthetic appeal. Success in a microscopy practical is itself inspirational, but it is not only the success of finding the microorganism which is satisfying — sometimes they look really attractive too! Sophisticated imaging techniques of microbial communities, or of components of individual cells are commonplace now, in textbooks, and on the web, and their aesthetic appeal may be overlooked. Images can be enhanced and modified, and find a diverse range of uses: calendars (*e.g.*, *Sf*AM 2007), websites, graphic art posters, clothing (www.iawareables.com), ceramics (*ASM News*, 2005, 71 (4), p 165), collage and so on (Figures 1 and 2).

Microorganisms can themselves be used to produce art. Alexander Fleming recognised the artistic potential of pigmented bacteria when he drew several cameos 'not written with ink but with bacteria which develop colours as they grow'. Since then, many budding artistic microbiologists have followed in his footsteps, drawing on agar plates with pigmented (*Microbiologist* Vol 3, No.4, December 2002) and bioluminescent species (*e.g.*, www.erc.montana.edu/bioglyphs/default.htm). Microscopic pictures made with diatoms were a Victorian interest, but production is still ongoing (www.diatoms.co.uk)!

Three-dimensional representations of microorganisms are not uncommon. Of course, models of toadstools can be found in many a craft shop, but more scientifically accurate models have been produced by bygone botanists and some are on display in the Whipple museum in Cambridge. The 20-sided polygon, the icosahedron, provides the maximum volume for the minimum building material, thus is an ideal structure for viruses and geodesic buildings such as the Eden project. The art of phage (www.phage.sdsu.edu/imagery) has also found exhibition space (Figure 3).

The consequences of bacterial and viral diseases, rather than the microorganisms themselves, provide ideal subjects for imaging the destruction wreaked by plagues through history (*e.g.*, www.wellcome.ac.uk), and to the more personal symptoms visited upon individuals (*e.g.*, *The Inheritance* by Edvard Munch). Stained glass windows have more recently provided a vehicle for symbolic representation of influenza pandemics (*e.g.*, Medical Library window, Royal London Hospital). The wide-ranging potential

Figure 1: Silk painting of Aspergillus fumigatus (Natasha Khan)

for using art to interpret or illustrate the significance of microorganisms was demonstrated to me in a University assignment where students produced, among other things: a Powerpoint presentation outlining the influence of plague on contemporary art through history; an artistic representation of the history of science; a collage in 1930s style of the importance of tuberculosis in literature; panels for the AIDS quilt; a profile of artists who interpret science through the medium of paint, etc (Figures 1 – 6) . The enthusiasm and talent of the students was really inspirational.

Figure 2: Notebook and jewellery inspired by microorganisms (Katy Lloyd)

Cross-disciplinary projects involving artists and scientists are common, and encouraged, particularly by the Wellcome Trust (Sciart) and other organisations (eg www.NESTAfuturelab.org). An exhibition, *Wonderful: visions of the near future* (www.wonderfulwebsite.net) sponsored by Wellcome, NESTA and the Arts Council, presented the discussions and realisations of artists and scientists working together collaboratively. One provocative piece, HeLa Hot (Christine Borland) related the imagery of HeLa cells to the person, Henrietta Lacks, from which the cells had originated. Discussions with artists at MMU have led to projects where the outcome has

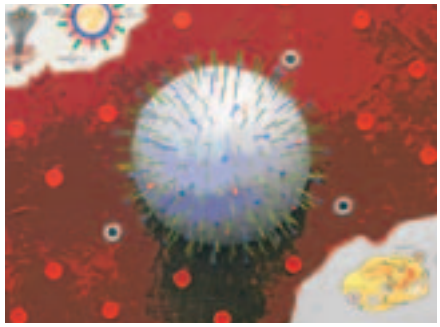

Figure 3: Three dimensional educational model of influenza virus, accompanied by cutaway hand model and powerpoint presentation (Nicola Barker and Rachel Herstell)

been artwork, exhibition, or installation, rather than scientific paper, report or presentation. Postgraduate art students have also used microorganisms and/or principles of microbiology as part of their project work. Undergraduates in art and biology together designed an installation for the foyer of a new science building. Although not successful due to the cost implications,

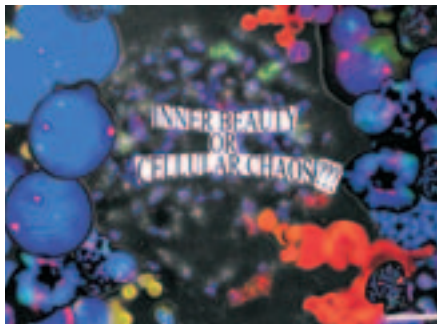

Figure 4: Images of micronucleus and chromosome damage using the FISH assay (Martin Curtis-Emerson and Anna Carlisle).

the work considered the importance of repeating, yet evolving structures in all aspects of science (fractals, DNA, evolution, polymers). The three dimensional prototype (Figure 6) reflects this flexibility within a more rigid framework.

It rapidly becomes apparent that the combination of microbiology with other subjects such as literature (5), where the impact of disease on the development of particular novels can be explored; or music (Helen Davies, University of Pennsylvania), where composition or lyrics can assist in recall of complex terminology, is easily possible. Subjects such as history, geography, politics, economics are

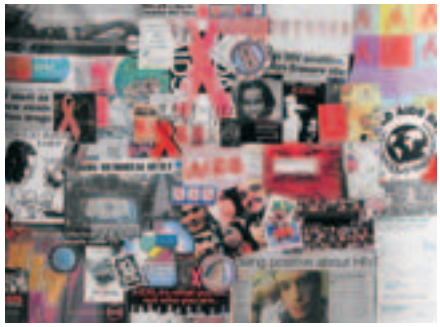

Figure 5: A collage of icons and images used to raise awareness of AIDS (Athena Chandni and Tramantza Bhagat)

inevitably associated with the epidemiology and management of emerging and re-emerging disease - thus whatever the focus of the audience, microbiology becomes of interest and relevance.

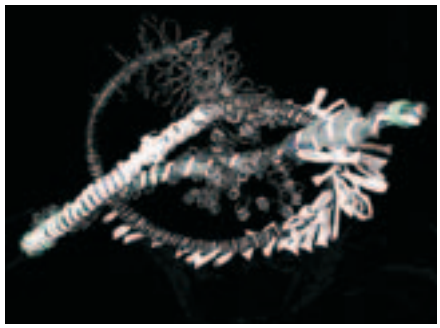

Figure 6: Prototype model for installation in foyer of Science Faculty

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Stat Note 8

In the eighth of a series of articles about statistics for biologists, **Anthony Hilton** and **Richard Armstrong** discuss:

Statistical power and sample size

There are two important questions that should be asked about any experiment. First, before the experiment is carried out, what sample size (N) would it be appropriate to use in a given situation? Second, what is the strength or 'power' (P') of an experiment that has been conducted, *i.e.*, what difference between two or more groups was the experiment actually capable of detecting?

The second question is of particular interest because an experiment in which a non-significant difference is reported confirms the null hypothesis (H_0) that no difference exists between the groups. This may not mean, however, that the hypothesis should actually be rejected because the experiment may have been too small to detect the 'true' difference. In any hypothesis test, the statistical test, *e.g.*, a 't' or 'F' test, indicates the probability of a result if H_0 were actually true and therefore, if that probability is less than 5% ($P < 0.05$), H_0 is usually rejected. The ability of an experiment to reject the hypothesis depends on a number of factors including the probability chosen to reject H_0 (usually set at 0.05), the variability of the measurements, the sample

size since larger values of N lead to more accurate estimates of statistical parameters, and the effect size, i.e., the size of the actual effect in the population, larger effects being easier to detect. Statistical software is now available to calculate P' and to estimate N in a variety of circumstances and it is therefore important to understand the value and limitations of this information. This Statnote discusses statistical power and sample size as it relates to the comparison of the means of two or more independent groups using 't' tests or analysis of variance (ANOVA).

How to calculate sample size for comparing two independent treatments

In Statnote 2 (Hilton & Armstrong 2005) we described an experiment to investigate the efficacy of a novel media supplement in promoting the development of cell biomass. Essentially two sets of 25, 10-litre fermentation vessels were filled with identical growth media with the exception that the media in one of the vessels was supplemented with 10ml of the novel compound under investigation. The vessels were then inoculated with a culture of *Bacterium 'x'* and the fermentation allowed to proceed until all the available nutrients had been exhausted and growth had ceased. The dry weight of cells was measured in each flask. A good question might be how many flasks should actually have been used in this experiment?

As a first step, decide on a value δ that represents the size of difference between the media with and without supplement that is regarded as important and which the experiment is designed to detect. If the true difference is as large as δ, then the experiment should have a high probability of detecting this difference, *i.e.*, the test should have a high P' when the true difference is δ. Levels of P' = 0.8 (80%) or 0.9 (90%) are commonly used whereas levels of 0.95 or 0.99 can be set, but are often associated with substantial sample sizes. To determine N for two independent treatments, the following data are required:

1. δ the size of the difference to be detected
2. The desired probability of obtaining a significant result if the true difference is δ (Z_β)
3. (Z_β) obtained from 'z' tables
4. The significance level of the test (Z_α) usually P = 0.05)
5. The population standard deviation σ usually estimated from previous experiments

The formula for calculating sample size is:

$$N = (Z_\alpha + Z_\beta)^2\ 2\sigma^2/\delta^2 \text{----------} 1.$$

A worked example using this formula is shown in Table 1 and suggests that given the parameters listed, the investigator should have used N = 36 in each group to have had an 80% chance of detecting a difference of 10 units. Note that Z_α is based on a two-tail probability but Z_β is always based on a one-sided test (Norman & Streiner, 1993). This is because the tails of the two distributions representing the two media overlap on only one side.

What are the implications of sample size calculations?

This procedure is designed to protect the investigator against finding a non-significant result and reporting that the data are consistent with H_0 when in fact the experiment was too small. This suggests that a sample size calculation should always be carried out in the planning stage of an experiment. However, in reality, sample sizes are usually constrained by expense, time, or availability of human subjects for research and quite often a sample size calculation will result in an unrealistic N. Microbiologists would be surprised at the number of samples required to detect modest differences between two groups given the level of variability often encountered in experiments. Hence, sample size calculations may be an interesting adjunct to a study and may provide an approximate guide to N but should not be taken too seriously (Norman & Streiner, 1993). In addition, increasing sample size is only one method of increasing P'. Reducing the variability between replicate samples by using more homogenous groups or the use of experimental designs such as a paired or randomised block design and which eliminate certain sources of variability may also increase P'.

Table 1. Examples of sample size (N) and power (P') calculation for comparing two independent treatments

A) Sample size calculation (N)

Difference to be detected δ = 10 units

Standard deviation σ = 15 units

Significance of test P = 0.05, Z_α (from Z table at P = 0.05) = 1.96 (two-tail test)

Power of test say P = 0.80 and therefore P of not demonstrating an effect = 0.20

Z_β = (from Z table at P = 0.20) = 0.84 (one-tail test)

$(Z_\alpha + Z_\beta)^2\ 2\sigma^2/\delta^2$ = 3528/1000 = 35.28, say 36 per group

B) Power calculation (P')

Suppose in above example, the experiment had been carried out with 36 per group but the standard deviation had been 20 units not 15:

$Z_\beta = (\sqrt{}\ N.\ \delta/\ \sqrt{}2.\ \sigma) - Z_\alpha = 0.17.$

Hence, P of ***not*** demonstrating an effect = 0.43 (from Z table) and therefore, experiment has a P' = 0.57 (57%) of demonstrating a difference of 10 units

Calculation of P'

Sample size calculations also contain a useful corollary, calculation of the strength or power (P') of an experiment to detect a specific difference. This type of calculation is very useful in experiments that have failed to detect a difference the investigator *thought* was present. In such circumstances, it is useful to ask whether the experiment had sufficient P' to detect the anticipated difference. To calculate P' of an experiment equation 1 is rearranged to give Z_β:

$$Z_\beta = (\sqrt{}\ N.\delta/\ \sqrt{}\ 2.\sigma) - Z_\alpha \text{ ---------- } 2.$$

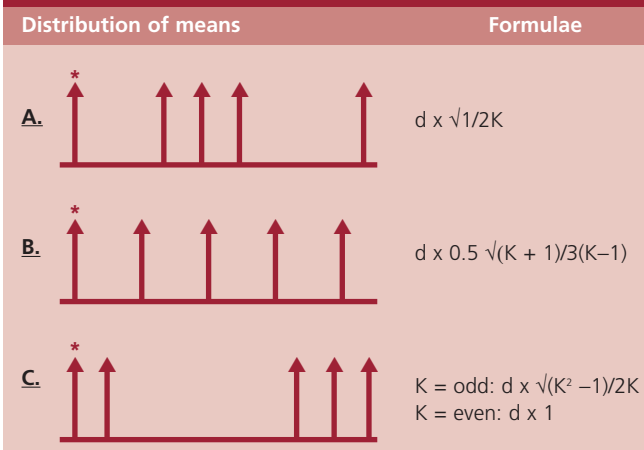
A worked example utilising this equation is given in Table 1. Suppose that the experiment described in the previous section had been conducted with a sample size of N = 36 but that the σ was actually 20 and not 10 units. The value of Z_β has fallen to 0.17 corresponding to a probability of not demonstrating an effect of P = 0.43. Hence, the probability of detecting a difference between the two means of 10 units has fallen to 57% and hence, P' would have been too low for this experiment to have had much chance of success.

Power and sample size in other designs

The equations used for calculating P' and N differ depending on the experimental design, *e.g.*, in a 'paired'

design (Hilton & Armstrong, 2005) or when comparing two proportions (Katz 1997). Statistical software is available for calculating P' and N in most circumstances and although the equations may differ, the principles described in this Statnote remain the same. However, the situation becomes more complicated if there are more than two groups in a study and if the data are analysed by analysis of variance (ANOVA) (Armstrong & Hilton, 2004).

Figure 1. Adjustment to the effect size for calculation of sample size in a one-way analysis of variance (ANOVA) (K = number of groups, * = control group)



Sample size and power in ANOVA

Calculation of P' is more complex when several group means are involved because the difference between the means may be distributed in various ways (Figure 1). An important statistic when several means are present is the effect size d = δ/σ where δ is the difference between the highest and lowest mean (Norman & Streiner, 1994). For example, if there are five groups (K = 5, a control and four treatments), one treatment may have a large effect while the remaining three may have similar but lesser effects (scenario A). In scenario B, the treatment means are spread more or less evenly and in scenario C, three treatments have large but similar effects and one has little effect and is therefore similar to the control. The essential approach is that 'd' is transformed into the effect size for ANOVA by multiplying by a formula which varies depending on the distribution of means. Various scenarios and sample formulae are illustrated in Figure 1 and how the calculations are made is shown in Table 2.

More complex experimental designs

In more complex experimental designs where there are many treatments or if a factorial arrangement of treatments is present, calculation of N by these methods becomes less useful. A more relevant concept is to consider the number of degrees of freedom (DF) associated with the error term of the ANOVA. In the general case, in a one-way design (Armstrong & Hilton, 2004) if there are 'p' treatments and N observations in each group, the error term will have p(N-1) DF and the greater the value of N, the greater the DF of the error term and the more precise and reliable the error estimate will be. A change of 1DF has a large effect on 't' or 'F' when DF < 10 but the effect is quite small when DF > 20. Hence, it is good

Table 2. Sample size calculation for a one-way analysis of variance (ANOVA)

Difference to be detected (largest mean – smallest mean) = δ
Assume individual means (K groups) equally distributed
Standard deviation = σ

1. Calculate effect size d = δ/σ

2. Adjustment to formula (from Fig 1): effect size for ANOVA = (f) = √d x 0.5 (K + 1/3(K – 1)

3. Look up 'f' in Table I (Norman & Streiner, 1993) to give sample size having chosen Z_α and Z_β

practice to have at least 15 DF for the error term and this figure will be dependent on both the number of treatments and N. In factorial designs (Armstrong & Hilton 2004), with different factors or variables in the experiment, the presence of factorial combinations of treatments leads to *internal replication* and therefore such experiments can often be carried out using much smaller sample sizes. The principles underlying factorial experiments will be discussed in more detail in a future Statnote.

Conclusions

Statistical software is now commonly available to calculate P' and N for most experimental designs. In many circumstances, however, sample size is constrained by lack of time, cost, and in research involving human subjects, the problems of recruiting suitable individuals. In addition, the calculation of N is often based on erroneous assumptions about σ and therefore such estimates are often inaccurate. At best, we would suggest that such calculations provide only a very rough guide of how to proceed in an experiment. Nevertheless, calculation of P' is very useful especially in experiments that have failed to detect a difference which the experimenter thought was present. We would recommend that P' should always be calculated in these circumstances to determine whether the experiment was actually too small to test H_0 adequately.

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Mark Reed explores the work of a a Technical Sales Representative: the essential link between the scientific commercial world and the laboratory

careers

European Technical Sales

Microbiology offers many careers in many different fields specialising in clinical sample testing, finished product, food sample testing and water testing, you can even test the structure of oil rigs for the effects of micro-organisms on them.

If we were to consider the total number of microbiology laboratories in testing, teaching and research, you can imagine that the number of tests being carried out is immense. In fact it is in the region of 1.2 billion tests per annum for the UK alone. Although there are many variants across the diverse field of microbiology testing, there are also many similarities, and one core similarity is that they all require commercially supplied kits, reagents, analysers, and disposable items. The technicians certainly wear the white coats and have the required qualifications, but they need an enormous array of products and services to help them do their job.

These products and services come from the scientific commercial world, and someone has to spread the word...the Technical Representative.

When I started my career in Microbiology, I had no intention of entering the commercial world, it was foreign to me. Having left school I started as a Junior "A" Medical Laboratory Scientific Officer (MLSO) and spent two years rotating through the disciplines in the pathology department of Arrowe Park Hospital on the Wirral (Microbiology, Haematology/Transfusion, Biochemistry and Histology/Cytology).

As you would expect in the most junior role, you get all the best jobs from labeling hundreds of samples to filing hundreds of reports, everyone has to start somewhere. The career structure at the time required a Junior "A" MLSO to select one discipline after this initial two years to specialise in one area. For me this was to be Microbiology and I spent three enjoyable years in the Wirral Microbiology Department based at Arrowe Park and Clatterbridge hospitals. Having successfully gained the required qualifications for state registration, I achieved the title of a Medical Laboratory Scientific Officer in Microbiology.

I then asked myself "where do I go now?" After much thought I decided to enter the commercial world. This started with observing a number of technical representatives calling on the laboratory to promote new products and services to the department heads, and also to service and support products being used in the laboratory. My first action was to collar one of these visitors and find out more. This resulted in a number of days travelling with a technical sales representative to a number of sites around the UK, and at this point I decided that this was for me. Although a difficult decision to make to leave the security of the laboratory, I had the comfort of knowing I would still be in touch with the industry that I enjoyed and I was merely crossing into an associated field. From there on I have never stood still.

Following an initial interview with a scientific recruitment agency (this is an essential starting point) I secured the post of Technical Representative with GIBCO Laboratories for the North West of England, specialising in the manufacture and supply of clinical microbiology media and associated products.

As the company developed over the years, so did the challenges available and within three years I held the position of European Industrial Business Development Manager for Europe, international travel became extensive and experiences became varied. Of course as careers develop, the need for training continues. Not only do we have to continually update our technical knowledge for our products and their practical application, we need numerous other skills such as negotiation, time planning, presentations skills, management and communication to name just a few. There are many companies that specialise in the training of technical representatives and all other levels of staff in commercial organisations and the continual learning process is as important as it is within the laboratory itself. My next move involved almost a chance encounter at an international trade show, where a company based in North America specialising in the development and manufacture of in vitro diagnostic kits and reagents for microbiology were considering establishing a subsidiary in the UK. So, there I was, 26 years old, married with two young children working for a large corporation with an opportunity to gamble all again and move to a brand new start up opportunity with all the associated risks. The potential of the challenge was the driving force behind my decision to take the opportunity at its starting point in Europe, now 18 years in, I realise it was a wise one.

If I were to offer advice to any reader about a career in technical sales, I would always emphasise that you must do what you enjoy most. As previously mentioned the world of microbiology is extremely diverse, one aspect being Technical Diagnostic Sales Representative into the Laboratory Industry, a role that I initially held myself. A typical day would be impossible to describe, as there are so many potential variants. What I will attempt to do here, is describe a potentially typical week with examples of the challenges and commitments that are required.

What are your key responsibilities as a Technical Representative?

They will include, but are not always limited to:

- Accepting responsibility for the efficient and profitable management of a sales territory.
- Assessing achievable market share for your products and achieving targets set.
- Being honest and truthful with yourself, your company, your products and your customers.
- Understanding and fulfilling your customers needs and those of your company.
- Reporting and assessing all activity on your territory.
- Understanding all aspects and capabilities of your products and being able to communicate them to your customers.
- Supporting all current business and handling all technical, service, and delivery enquiries.
- Reporting back to your company on your market and your customer's needs.
- Attending sales meetings, training sessions and seminars in house.
- Attending trade shows, conferences, lectures and discussion groups to represent your company and also to further your own education.

A typical Week

Like most jobs the week begins on Monday with an early rise, into the home based office, or if you are fortunate enough to live close to your company office, you can work from there. The first task is to check emails, post and faxes for any details that may alter your planning of the coming week and any appointments and commitments you may have. With a map of a large geographical area covered in a range of coloured markers, you now begin the task of contacting your customers by phone and making your appointments to a manageable travel plan. During this period you are interrupted constantly of course by the office contacting you with questions, your customers contacting you with questions, and the boss always wanting to know what you are doing. Finally you have in front of you a plan for the week ahead, with your hotels booked for overnight stays where necessary.

Monday: Planning, appointments, emails, prepare required literature packs, assemble catalogues, check samples and demonstration material. Complete required quotations, respond in writing to customers from previous work, update office on plan for week.

Tuesday: Five appointments

Wednesday: Five appointments and an evening discussion group to attend as the sponsor.

Thursday: Five appointments with a piece of equipment to install at 4.30pm on the last appointment.

Friday: Sales meeting at head office for new product launch.

With all preparations made, the week continues with an early departure on Tuesday to arrive on time for your first

appointment allowing for traffic issues and parking.

Appointment one: the first call involves a well-known customer who has used your products for many years and always welcomes you to discuss new developments in your product range. Everything is satisfactory with the products being used, stocks are sufficient at present, no performance issues have been raised and deliveries are arriving on time.

Today you have a new product that you believe will offer an improvement over the current method being used by eliminating equipment requirements and waiting time for results. Your presentation goes well covering all technical aspects of the product and you suggest an actual demonstration of the product in the laboratory for your customer's colleagues at a later date. Having checked your diaries, a date is

set for the following Friday, when you will return to perform the demonstration to the laboratory staff and a presentation covering the science behind the technology of the product. This later develops into a lunch time discussion group and you agree to perform a powerpoint presentation prior to the laboratory demonstration.

On returning to your car, provided by your company of course, you find a missed call from the office on your mobile phone and a voice mail to contact a customer urgently. The office are asking for guidance on pricing that is required for a tender document that has been received from your customer base. This takes up approximately 20 minutes of your time as you work with your colleagues to ensure the tender is as required. The customer, who has left

a message for you to contact him, left no details as to what the enquiry may be. Your first task is to check your files to familiarise yourself with details of your last meeting with the customer, and to check with the office to ensure there are no issues with the customer that may help you. On contacting the customer you learn that they have a problem with the interpretation of some results with a product supplied by yourselves. Having offered advice by telephone, you ascertain that you will need to visit the customer as soon as possible and agree to do this the following Tuesday as requested by them.

Having now dealt with two unexpected interruptions, you are running 40 minutes late for your next appointment. A few courtesy calls to your customers resolve any potential inconvenience as you shuffle the times around to suit all.

Appointment two: offers a challenge where you have previously performed a demonstration of your new product, and your customer is now ready to negotiate the terms under which you may potentially supply the product. The staff in the laboratory were impressed with the product evaluation to the extent that they have requested a change. Negotiations are completed and all that now remains is for you to confirm all details in writing. You advise your customer you will do this via email that evening such that he has it for the following morning and can arrange matters with his purchasing department.

Appointment three: following on from successful discussions concerning some of your disposable products, you have the opportunity to arrange samples to be sent to the customer for evaluation. This you do by telephone once you have left the customer, you then contact the customer again to confirm that the samples will arrive the following day and that you will contact him a few weeks to discuss their evaluation.

Appointment four: on arrival your customers has been called to an urgent meeting and is unable to see you. At your request one of his colleagues steps in for a few minutes and you ascertain that all is well with the products that you are supplying, and make arrangements to return the following Friday, this you will confirm by email. Back to the car now with perhaps 30 minutes to spare that you will use to call customers you saw last week to confirm arrival of quotations, technical details, promotional details and samples that you arranged.

Appointment five: much the same as appointment one and very satisfying.

With a day completed, all that remains is to check your messages, write up your reports, set a list of action points following the commitments that you have made with your customers, and start what may be a long journey home depending on the traffic. If you are staying over night in a hotel, a relaxing time awaits before you start a little paperwork in the evening, this of course you would also do if you were travelling home.

Your week now progresses well as you work through your appointments, and gradually build your business portfolio on your territory. Your last appointment was particularly satisfying as you have successfully installed a unit of your latest equipment in a laboratory on loan for two weeks. All of the laboratory staff appeared very excited at the prospect of the department head allowing this, and perhaps finding funding for its purchase. This latter point of course is another area where your skills, patience and professionalism will be required as you will now also be dealing with the Estates Management, the Purchasing and the Medical Devices departments within a hospital.

On Friday you are required to attend head office for a sales meeting in the morning where you will be required to present your sales results for your territory over the last 3 months. You will also offer a comparative analysis to the same period last year and submit forecasts for the coming three months with details of all evaluations, samples, quotations and tenders currently active or underway.

During the afternoon you will attend a training session presented initially by the product manager, concerning a new product that is being launched next month, and then with the marketing manager to plan the launch on your territory.

Whilst all of this is going on in your day, many other support roles are continuing behind you as back up. Many of these roles offer career path opportunities, and all offer service, support and a future to your customers. These will include customers services management, dispatch management, sales and marketing management, technical support, regulatory affairs, production and perhaps of most interest, certainly to your customer, product research and development based on new emerging technologies and customers market requirements.

Technical Sales is a challenging and rewarding career and offers many opportunities for career progression. Technical Sales Representatives are an essential link between the scientific commercial world and the laboratories, and a professional technical relation between both is essential. No day will ever be the same, and no career will ever follow a set path.

Mark Reed, General Manager
Pro-Lab Diagnostics Europe

Students into Work Grant reports

information

am I eligible - can I apply?

If you are FULL member who can offer an undergraduate microbiology student work during their summer vacation. If you would like to read about the experiences of Students who have benefited from this Grant, you can do so in each issue of *Microbiologist*. For Further information visit: **www.sfam.org.uk/grants.php**

guidelines

1. Any full member of the Society who can offer an undergraduate student, or a recent graduate (within three months of graduation), a work placement is eligible to apply for this grant. The placement can last up to a maximum of 10 weeks.

2. The Grant will normally provide support for the student at the rate of £160 per week and up to £500 for specified research costs, e.g., consumables used by the student. The monies will usually be paid to the Department in which the student/graduate works.

3. Applications should be made by the supervisor using the 'Students into Work' PDF application form provided on the website or the paper form obtainable from the Society Office.

4. Applicants must provide details of the intended project as well as information about how the project will benefit the student.

5. Successful applicants and their students/graduate must write a report on the placement. This may be published in *Microbiologist*.

6. Normally a member may not apply for a further grant until a period of two years has elapsed.

7. There is no closing date for this Grant and applications can be made any time during the year. Applicants must apply at least 6 weeks before the proposed start date.

The Impact of Environment upon the Metabolome of *E. coli* and *L. monocytogenes*

Between the second and third years of my BSc Microbiology degree at The University of Nottingham I was offered a ten week research placement. This took place within the University of Nottingham Food Microbiology laboratories, and was generously funded by the Society for Applied Microbiology. The placement involved a metabolomic study of *Listeria monocytogenes* and *Escherichia coli*, allowing me to gain experience of an emerging area of microbiology.

Metabolomics is a new tool for the microbiologist, allowing a snap-shot of the pool of intracellular metabolites to be taken. This then allows for comparison of the metabolic profiles of bacteria grown under different conditions to identify any changes that take place. Although still in its infancy, the potential of metabolomics is vast. It has already been used to discriminate strains of pathogenic *Bacillus cereus* (Bundy *et al.*, 2004) and for profiling of *Cryptosporidium* in water (Ruecker *et al.*, 2005). Possible applications include microbial forensics and drug discovery (Jaki *et al.*, 2006). However, despite these current and possible applications, data on the impact of altered environments on the metabolome of microorganisms is still relatively scarce. It is data of this kind I set out to gather during my summer project.

An avirulent *Listeria monocytogenes hlyA EGD*, deficient in the haemolysin gene, and *Escherichia coli* W3110 were used in this study. Starter cultures were first prepared by incubation in Brain Heart Infusion broth (BHI) for 24h. Cells were then subjected to a low nutrient stress and to a moderate thermal stress. This was achieved by transferring cells from BHI into Maximum Recovery Diluent (MRD), either alone or supplemented with Sucrose. The effects of various incubation temperatures were also studied. The aim of the work was to identify specific compounds that were characteristic for these different environmental conditions.

After incubation, the cells were

Table 1. Examples of compounds that differed between *E.coli* and *Listeria monocytogenes*

Ion[a]	Time[b]	[c]Compound	Probability value
88	5.35	acetoin	0.0082
59	20.89	octanoic acid	0.0008
117	25.1	indole	0.0012
129	26.89	decanoic acid	0.0112
60	37.1	tetradecanoic acid	0.0026

[a]Ion is the key ion used to preliminarily identify compounds. [b]Time is retention time from GCMS. [c]Compound represents the identity of the compound. [d]Probability value represents the statistical significance of the difference between E.coli samples and L.monocytogenes samples (by Students T-test)

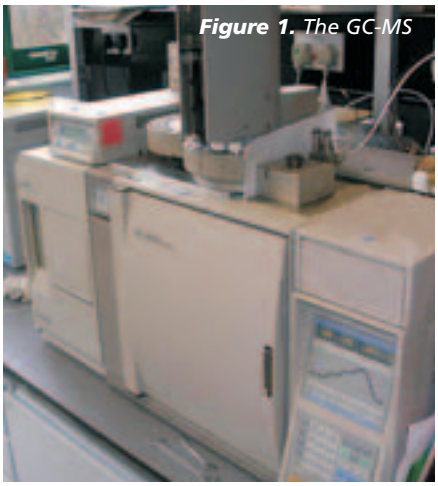
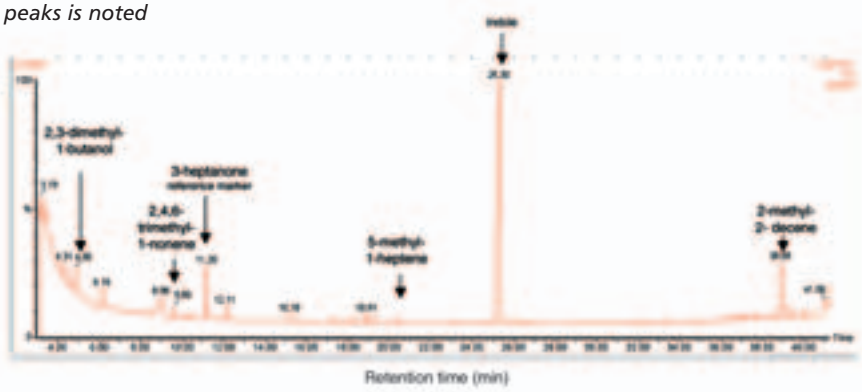


Figure 1. *The GC-MS*

sonicated on ice to release their intracellular metabolites. Dichloromethane was added to dissolve the organic compounds released from the cells, and the mixture was centrifuged to yield a separate DCM layer containing the metabolites. This DCM layer was concentrated by evaporation under a flow of nitrogen gas, transferred into vials and analyzed using Gas Chromatography with Mass Spectroscopy (GC-MS) (Figure 1). All of the samples were spiked with a known volume and concentration of 3-heptanone to act as an internal control.

Figure 2. *Example chromatogram obtained using GC-MS. Sample shown is from E.coli incubated at 42 °C in MRD without sucrose. The probable identity of some key peaks is noted*



The chromatograms produced were used to identify and quantify compounds in the DCM extract of intracellular metabolites (Figure 2). Firstly, a computer programme was used to generate a spreadsheet containing information on the retention times of all of the ions in the samples. This data was then used to identify clusters of ions at a constant retention time, indicative of a single compound. The ion and retention time information was used to isolate the relevant peak in the chromatogram set, allowing them to be integrated to yield peak areas by another computer program. A second spreadsheet was subsequently produced, containing the areas of peaks of interest in all of the samples tested. Basic statistical tests are in the process of being applied in order to determine the differences between the samples.

Considering the complexity of the intracellular metabolome, even studying a subset of it as we did here, has generated an enormous and complex dataset that we are still evaluating statistically. Metabolomic differences were observed between *Listeria monocytogenes* and *Escherichia coli*, with sixteen compounds showing a consistent statistical difference. Table 1. gives details of some of these compounds. The results indicate that extraction and analysis techniques were successful, and that the method we used is efficient in detecting metabolite level changes. Initial analysis also shows that there are differences between the two bacteria grown under different environmental conditions, but it has proven difficult to achieve reproducible results with the growth conditions used.

Throughout this placement I felt I was building on the knowledge and skills already gained during my undergraduate degree. I have learnt new techniques, such as metabolite extraction and the use of GC-MS. I have also gained a greater understanding of how a microbiology laboratory operates, and I understand how much preparation takes place before teaching practicals, that I had once taken for granted!

The placement has taught me many transferable skills that I feel will help me in my future career. This includes skills specific to microbiology, but also more generally time management, organisation and communication skills. My Information Technology skills have also vastly improved, and I am far more confident in using IT for data transfer and analysis. I am very grateful to Dr. Tim Aldsworth and the S*f*AM for giving me the opportunity to undertake this placement. My thanks also go to Professor Andy Taylor and Dr Rob Linforth for use of the GC-MS equipment and for their invaluable assistance.

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Evaluation of the effectiveness of contact plates for monitoring a microbial environment

Hospital acquired infections due to opportunistic pathogens are an important problem in the healthcare environment. Many microorganisms can survive drying on inanimate surfaces, contributing to an increased rate of microbial transmission to patients. The provision of sterile pharmaceutical products made *in situ* in hospitals is particularly challenging, and the absence of microbial contaminants from surfaces and their monitoring and control are essential. There are many control tests to evaluate surface contamination. A commonly employed protocol makes use of Contact Plates. This method is simple and mostly useful for sampling flat and smooth surfaces. It consists of pressing a contact plate, containing sterile agar with or without neutralizers, on the surface for 10 seconds and then incubating the plate to allow the growth of any surviving microbial contaminants. Unfortunately there is little documented evidence to support the efficiency of this technique in assessing the actual level of surface contamination. The aims of this project were to validate a quantitative sampling technique and compare the efficiency of three different commercial contact plates used to monitor the microbial environment.

Staphylococcus epidermis (NCIMB8853) was the biological indicator used for this project. It was grown daily on TSA slopes at 37°C.

The counting procedure was validated in order to assess the number of bacteria in the suspension used. The procedure consisted of serially diluting the resuspended slope and plating three drops (10μl) of the highest dilutions on TSA plates. Three replicates were prepared for each suspension. After 24h incubation at 37°C, the number of colonies for each drop was counted and the number of colony forming units (cfu) in the original suspension calculated.

Stainless steel surfaces were investigated in this study. Before using contact plates, I validated the bacterial recovery from stainless steel discs. One drop of 10μl of bacterial suspension (1-4 x 10^6 cfu/ml) was inoculated at the centre of a steel disc and dried for 1h under a laminar air flow cabinet. The surface of the disc was then flushed 12 times with 10ml of buffer (tryptone sodium chloride). Ten drops (10μl) were then plated on the surface of a TSA plate and the colonies / drop were counted after 24h incubation at 37°C. The % of recovery was calculated as: (CFU/ml measured / CFU/ml expected (i.e. control)*100. For validation purposes, this protocol was repeated ten times. The experiment showed a recovery from the steel disc of 71.4% of the original suspension.

Contact plates were obtained from Biomerieux (Count-Tact Irradiated Agar; France), Cherwell (T/V Contact Plate; UK) and Oxoid (Tryptone soya agar contact plate; UK). The surface of three sterile stainless steel sheets (of identical grade and finish to the discs) was divided into a four box grid. In each box, four drops (10μl) of a known bacterial concentration were inoculated. Different contact plates were randomly assigned to each of the 12 boxes. An applicator was used to standardise surface testing by applying a uniform pressure of 500gr on the surface of the agar plate for 10 seconds. Then, contact plates were incubated for 24h at 37°C and colonies counted. The three commercial brands showed significantly different recovery. Cherwell contact plates showed the highest recovery rate with 39%, whereas the Biomerieux and the Oxoid recovered 27% and 23% bacteria, respectively. Cherwell contact plates, even though they show a high recovery rate, were less reliable due to a high variability in results.

Other parameters were measured, such as loss of water, wetness of surface, presence or absence of neutralizers, and fertility. Loss of water after incubation was higher for the Oxoid plates. Regarding wetness of surface, I observed that when the agar surface was more moist, contact plates had a better adherence to the surface, possibly allowing dried bacterial inoculum to inoculate the agar more effectively. Finally, the presence or absence of neutralizers within the agar did not make any difference to recovery. Fertility was measured by inoculating the diluted resuspended agar slope directly onto the contact plates, which were incubated at 37°C for 24h. There was no difference in fertility between the different plates.

I also investigated the effect of expiry date on recovery efficiency of the Biomerieux contact plates, which did not affect the recovery from the plates.

Furthermore, we decided to use another biological indicator. I investigated the recovery of *Staphylococcus aureus* (NCIMB9518) on stainless steel sheets, using the same protocol as above. The recovery was significantly higher than with *S. epidermis* for the Biomerieux and Oxoid contact plates. However, the Cherwell contact plates showed a similar recovery for the two micro-organisms.

The objectives of this project were met and interesting results were observed. Contact plates, which have an important role in monitoring microbial contamination of the environment, did not appear completely reliable with an overall low recovery observed when a validated protocol was used. More importantly differences between brands were observed. Underestimating the microbial contamination of surfaces might have consequences on the quality assurance of pharmaceutical sterile products made *in situ* in hospitals.

I would like to thank my supervisor, Jean-Yves Maillard, and in particular SfAM for funding this short-term project, which provided the opportunity work in a microbiology laboratory and to acquire new skills. I am also grateful to my colleagues, who helped me with my project and made the work very pleasant. I have since decided to undertake a PhD in microbiology.

Federica Pinto

The President's Fund reports

information

am I eligible - can I apply?

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4. Completed applications must include an abstract of any intended contribution to be made at the meeting and must be received by the Society not less than six weeks before the date of the event.

5. Student member applications must be supported by their supervisor and include the contact telephone number(s) and email address(es) of the supervisor or head of department who is supporting their application.

6. The maximum grant available is normally £1,000.

7. Under exceptional circumstances this maximum may be exceeded.

8. The award of this grant is at the sole discretion of the Honorary President of the Society for Applied Microbiology.

9. The applicant must write a short article of between 500 - 1,000 words within four weeks of the meeting, the content of which will be agreed with the Editor of SfAM *Microbiologist* and will be published in the magazine at the discretion of the Editor. Photographs of the applicant and/or the subject of the article are desirable. These should be supplied as (a) digital files at a size of not less than 4 inches square at a resolution of not less than 300 pixels per inch, or (b) original photographic prints which will be scanned and promptly returned to the applicant.

Investigating the role of freshwater epilithic biofilms in harbouring virulent *Escherichia coli* O157 in an agricultural environment

***E. coli* O157 is a relatively new** addition to the number of pathogenic intestinal bacteria commonly isolated from both humans and animals. It first came to prominence around 25 years ago and has since become the subject of a great deal of research, suspicion and even fear among the lay and scientific communities. It often makes the headlines as the etiological agent behind food poisoning outbreaks, and has even been referred to as the 'hamburger bug'. However, its role as a waterborne pathogen is much less well publicised, and outbreaks of waterborne *E. coli* O157-associated disease rarely make the same media impact as that of, for example, cholera.

E. coli O157 is a medically important *E. coli* strain, its characteristic feature being the expression of two verocytotoxins (VTs) and the protein, intimin, which are associated with incidents of both haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). The verocytotoxins are also known as Shiga-like toxins because they share 97% amino-acid sequence homology with the *Shigella dysenteriae* type 1 toxin (Paton & Paton, 1998), which is the causative agent of bacillary dysentery. *E. coli* O157 is not, however, the only serotype known to express these verocytotoxins. It has been reported that numerous 'O' and 'H' serotypes, and combinations of serotypes, may express the toxins either singly or in addition to other virulence factors such as intimin or an enterohaemolysin. Verocytotoxigenic (VTEC) serotypes recovered include: 02:K, 0103:K-, 0104:K, 0128:K, 0153:K- and O157:K-:H7 (González & Blanco J, 1989).

E. coli O157 has been isolated from private and municipal water supplies, mainly as a result of contamination of the source water. Animal grazing and the use of manure-based fertiliser can lead to an influx of faecal bacteria to the soil, where cultivation and natural run-off, as well as ground and surface waters, transfer the bacteria deep into the soil.

Here at Brighton University we have been researching the ability of freshwater epilithic biofilms to harbour *E. coli* O157 that possess virulence factors associated with incidence of human disease. A mixed farm (containing arable farming with animal husbandry) situated on the South Downs in East Sussex, UK, was selected for the field studies. Devices using indigenous flint shingle were set up at four sites along the stream network to allow natural biofilm formation. Stones were removed from each site at four week intervals for one year, and screened to characterise eight *Enterobacteriaceae* genera and obtain total heterotrophic counts. Pooled faecal samples were obtained from indigenous grazing animals and characterised to investigate their potential role as vectors of this pathogenic organism. Using the

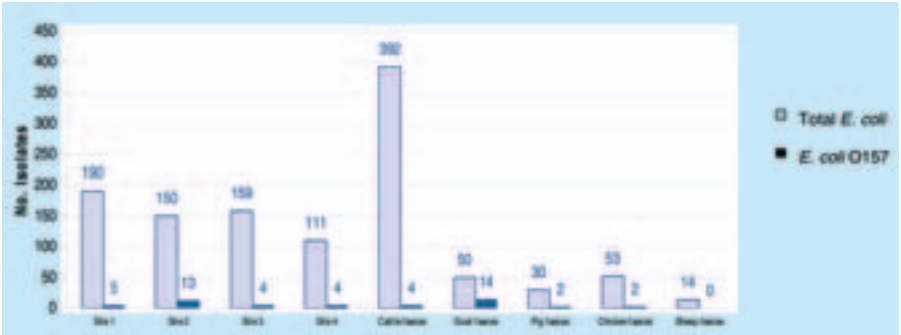

Figure 1. Summary of the total E. coli and E. coli O157 isolates recovered from each site sampled; E. coli O157 population presented as a portion of the total E. coli number (sites 1-4 represent the four biofilm locations).

PhenePlate™ technique, all of the *E. coli* isolates were analysed for kinetic biochemical phenotypes. The presence of four virulence genes associated with *E. coli* O157-associated disease were identified using PCR. The genes tested for were the stx1 (verocytotoxin 1), stx2 (verocytotoxin 2), *eaeA* (intimin) and *hly* (enterohaemolysin) alleles.

Phenotypic analysis revealed that distinct sub-populations of *E. coli* exist for each animal population, some of which displayed a significant phenotypic similarity to those recovered from the biofilms, suggesting a common source. Of 1002 *E. coli* isolates recovered from biofilms and animal faeces, 48 were confirmed as the O157 strain by latex agglutination. Sub-populations of *E. coli*, including *E. coli* O157, that demonstrated significant phenotypic similarity with animal faecal isolates (T-test, P = 0.05) were isolated from the biofilms, perhaps suggesting a common origin. The stx_2 gene was the most frequently isolated single gene (31 isolates), while stx_1 was the least frequently recovered (3 isolates). These three stx_1^+ *E. coli* O157 isolates were recovered from biofilm matrices but none were recovered from the pooled faeces of grazing animals. They displayed a high phenotypic similarity to each other, perhaps suggesting that the isolates may originate from the same source or host, although a reservoir was not determined in this investigation. In cattle it has been reported that stx_1+ strains are superseded by stx_2+ ones as their hosts mature. It is possible that these isolates originate from calves that have matured and that their gastric flora has been replaced or that an environmental reservoir exists for this strain that was not identified during the course of this study, such as soil or other sediments.

Epidemiological studies have often reported that an environmental reservoir may allow for the reintroduction of a pathogen to a susceptible host population to cause additional disease after an initial epidemic or outbreak has subsided. Cholera is a major problem concerning the rivers of the Indian sub-continent, and a series of *Vibrio cholerae* O139 outbreaks in 1992 lead scientists to believe that this was the start of the eighth pandemic of this disease. Within ten months the strain had spread to neighbouring countries, having replaced indigenous *V. cholerae* strains, and to be itself replaced by the O1 strain by 1996.

This situation suggests that a strain can come to dominate regions inhabited by closely related strains and cause widespread disease in susceptible populations. Hence the presence of viable pathogenic *E. coli* O157 isolates in temperate freshwater biofilms is a public health concern and this research should be followed up.

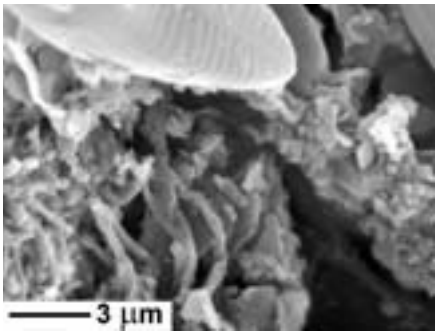

Figure 2. An electron micrograph of a freshwater epilithic biofilm

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In the UK, HUS is the most common cause of acute renal failure in children (Hunter, 2003). An understanding of the behaviour of the causative bacterium in the environment is important to the prevention of future cases of disease. The presence of viable and virulent *E. coli* O157 isolates in aquatic biofilms represent a potential hazard to human health. Although aquatic biofilms may not be a fundamental component of the life cycle of human pathogens such as *E. coli* O157, biofilms are increasingly recognised as an important reservoir of pathogenic bacteria. This work demonstrates that *E. coli* O157 related to agricultural animal populations are able to exist within aquatic epilithic biofilms and retain the potential to cause disease in human populations.

Acknowledgments

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Anti-microbial activity in traditional milk fermentations of Southern Africa

The presence of coliform bacteria in milk products generally provides an index of the hygienic standards of the product. In addition, coliforms present in the milk product may include pathogenic strains of *Escherichia coli*. Lactic acid bacteria in fermented dairy products inhibit *E coli* through the resulting low pH and by producing anti-microbial compounds including hydrogen peroxide, lactic acid, volatile fatty acids, bacteriocins and bacteriocin-like peptides (De Vuyst & Vandamme, 1994). However, survival of *E.coli* in fermented dairy products is highly variable, depending on the species of lactic acid bacteria, amount and rate of lactic acid production during fermentation, and storage temperature of the product after fermentation.

In Zimbabwe and other countries in the Southern African region, rural communities produce naturally fermented milk 'Amasi', which forms a major part of their diet. Unpasteurised cow's milk is allowed to ferment spontaneously in an earthenware clay pot or gourd for two to three days at ambient temperature, and the microbial flora responsible for the fermentation derived mainly from the raw milk and the walls of the container. Fermentation is dominated by lactic acid bacteria, typically in the order of 10^8 CFU/mL (Beukes *et al.*, 2001). Species isolated from Amasi and other traditional fermented milks in previous studies include *Lactobacillus plantarum*,

Lactococcus lactis, *Leuconostoc lactis*, *Lactobacillus delbrueckii* and *Lactobacillus helveticus* (Feresu & Muzondo,1990; Beukes *et al.*, 2001; Mathara et al.,2004).

It is necessary to know the behaviour of pathogens during manufacture and storage of fermented dairy products since it is possible that pathogenic bacteria may gain access to these products before, during and after fermentation. The purpose of this study was therefore to determine the fate of pathogenic and non-pathogenic *E.coli* during fermentation of traditionally fermented milk and LACTO (an industrial equivalent prepared from pasteurized milk and mesophilic starter cultures), during the fermentation process and storage of the fermented products at ambient and refrigeration temperatures.

The two pathogenic strains (104/8 and 0125K70) used in the study were human strains obtained from the Public Health Laboratories, Parirenyatwa Hospital, Harare. The non-pathogenic strain (NP1620) was a stock culture maintained at the University of Zimbabwe, Department of Biological Sciences. Sufficient of each of the 24 hour broth cultures of the three *E.coli* strains were added to unpasteurised, pasteurised and freshly inoculated 'LACTO' milk samples to give approximately 10^3 *E.coli* cells/mL. All the milk treatments were left to ferment at ambient temperature for 24 hours, after which one set was stored at ambient temperature, while the other set was refrigerated at 5°C for a further 96 hours. The pH, percentage lactic acid, and numbers of *E.coli* were determined at 24 hour intervals. Lactic acid bacteria were identified to species level according to sugar fermentation reactions (API 50 CHL Biomeriéux, Marcy-L'Etiole, France), and preliminary identifications confirmed by PCR analyses.

Both pathogenic strain 104/8 and non-pathogenic strain NP 1620 decreased in numbers by 2 logs in the first 24 hours of 'LACTO' fermentations and could not be recovered after a further 24 hours at 5°C. Strain 0125K70 could be recovered after 48 hours, although there was a 2 log reduction in numbers (Figure 1). All three *E. coli* strains multiplied by 3-6 logs after 24 hours at 20°C during traditional fermentation of

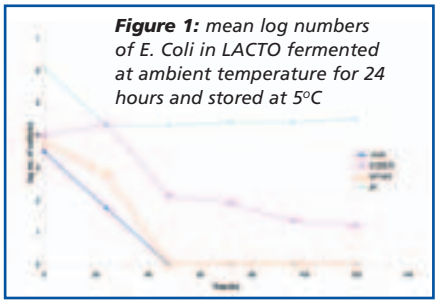
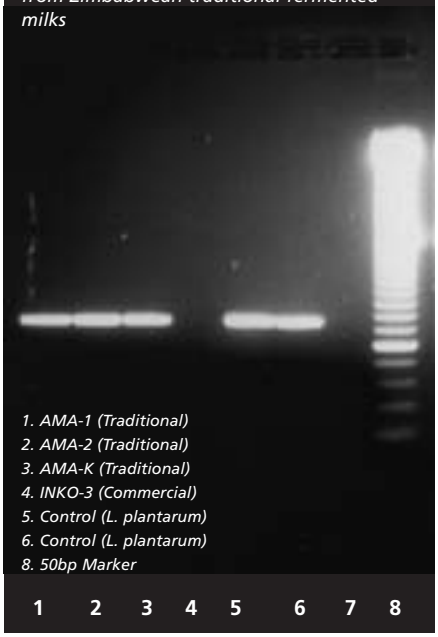


Figure 1: *mean log numbers of E. Coli in LACTO fermented at ambient temperature for 24 hours and stored at 5°C*

unpasteurised milk and to an even greater extent by 5-7 logs during traditional fermentation of pasteurized milk. However, numbers declined after the initial 24 hours of fermentation by up to 3 logs after a further 48 hours storage at ambient or refrigeration temperatures.

Figure 2: PCR band patterns of LAB isolates from Zimbabwean traditional fermented milks



Lactobacillus plantarum was shown to be dominant in the naturally fermented milk samples (Figure 2), while *Lactococcus lactis* was also isolated from the Amasi samples under study. A cell-free supernatant containing bacteriocin from strain AMA-K identified as *Lactobacillus plantarum* also inhibited the growth of *Listeria innocua Enterococcus faecalis*, *Lactobacillus casei*, *Lactobacillus sakei*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. The bacteriocin was determined, based on Tricine — SDS PAGE to be 2.9 kDa in size, and was also shown by atomic force microscopy to have a bacteriolytic mode of action (Todorov & Dicks, 2004).

LACTO fermentation was more inhibitory to *E. coli* strains than the natural fermentation of raw milk, probably due to the more rapid acidification by industrial starter cultures. Although bacteriocin production in natural milk fermentations by LAB is inhibitory to the growth and survival of *E. coli* and other pathogenic species, the *E.coli* strains under study were not eliminated by the fermentation process. High standards of hygiene should therefore be observed during milking and fermentation of milk to minimize potential health hazards from enteric pathogens, particularly where production is to be scaled up for commercialisation.

I would like to thank the National University of Science and Technology, Zimbabwe, SARBIO and the National Research Foundation (South Africa) for financial support and SfAM for an award from the President's Fund for attendance at the Food-Micro 2006 conference in Bologna, Italy.

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Bacillus - ACT 2007

Oslo, Norway, June 17-21 2007

The International Conference on *Bacillus anthracis, B. cereus*, and *B. thuringiensis* (Bacillus-ACT2007) will be held in Oslo, Norway, June 17 - 21 2007

These bacteria comprises a genetically closely related group of gram-positive bacteria exhibiting a highly divergent range of phenotypic properties. Many bacteria classified as *B.cereus* are widely distributed in the environment, probably with reservoirs in the soil and as commensal inhabitants of the intestines of insects. Occasionally they cause food poisoning and soft tissue infections particularly of the eye. Other members of the group are classified as *B.thuringiensis* and are primarily insect pathogens which like *B. cereus* can occasionally cause human infection. A third pathogenic phenotype is exhibited by *B.anthracis*, which infects mammals and occasionally humans, and has gained notoriety as a consequence of its use as a bio-weapon.

The diversity of phenotype within this group, often mediated by plasmid encoded factors, has resulted in the establishment of distinct, organism specific, research communities focusing on issues such as food spoilage, the development of bio-pesticides, animal and human health and bio-defense. Given the common genetic background of these organisms a major aim of **Bacillus-ACT2007** is to facilitate the exchange of ideas between these different communities. The conference, under the chairmanship of Dr Anne-Brit Kolsto, represents a fusion of two meetings, the International Conference on Anthrax, and the International Workshop on the Molecular Biology of *Bacillus cereus, B. anthracis*, and *B. thuringiensis*. The mission of the conference, which is being supported by a generous grant from S*f*AM, is to bring together researchers involved in scientific research related to the physiology, genetics, genomics, molecular biology, and pathogenesis of this group of bacteria. The deadline for the submission of abstracts was the 15th February while registration opens on 1 April and closes 4 May.

Further details can be obtained by visiting the website at: http://bacillus-act07.uio/act07/

Les Baillie, University of Maryland Biotechnology Institute, Baltimore, Maryland

corporate news

The latest news, views and microbiological developments from our corporate members

Lab M introduce Fraser Broth^{PLUS}

Fraser Broth^{PLUS} and **Half Fraser Broth^{PLUS}** are recently introduced variants of Lab M's standard **Fraser Broth** medium for the isolation of *Listeria* species. The new media are formatted to give improved results in both traditional culture and ELISA methods. In common with all Lab M's **Fraser Broth** range, they meet the specifications of the ISO 11290 standard for the detection and enumeration of *Listeria* monocytogenes. **Fraser Broth** is traditionally used as a secondary enrichment medium for the isolation of all *Listeria* species. With the incorporation of half strength supplement, it can also be used as a primary resuscitation or enrichment medium.

Fraser Broth^{PLUS} and **Half Fraser Broth^{PLUS}** differ from conventional formulations in the inclusion of selective agents in the base medium. The ferric ammonium citrate (FAC) component is provided as a supplement to be added after sterilisation. This format has been shown to give improved selectivity with pure cultures and food samples. The option to omit FAC from the final medium is advantageous where ELISA techniques are used, reducing the incidence of false positive results.

Both new media are tested in accordance with, and meet the technical specifications of ISO/TS 11133-2 standard for the microbiology of food and animal feedstuffs.

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AFNOR expert status for CCFRA

There are many test kits for food microbes. But how does a food company know whether a particular kit meets their needs? This is usually addressed by looking at the kits validation status — in other words, whether it has been put through its paces by an independent laboratory as part of a recognised scheme. In 2006 **CCFRA** was awarded expert laboratory status by AFNOR (Association Française de Normalisation), a major European method validation scheme. Dr Roy Betts, Head of Microbiology comments:

"*The food industry can have confidence in kits validated by AFNOR, as the validation has to conform to BS EN ISO 16140. As an expert laboratory, we are charged with implementing the validation to these exacting standards. This includes comparing the performance of the kit with the established reference method — for example, performance of kits for Salmonella would be compared with EN ISO 6579. We can now carry out validation under a range of schemes including AFNOR, AOAC International, Microval and Nordval, as well as bespoke validation for kit manufacturers. This recognition of our capabilities is a gratifying reflection of CCFRA's world standing in analytical microbiology.*"

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Bio-Rad launch SaSELECT

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Invitrogen launch new Application Notes

Invitrogen Environmental Diagnostics (formerly **Dynal Biotech Microbiology**) has recently launched a new series of Application Notes which highlight new or interesting product applications or developments. The Application Notes are written by customers or research and development scientists. Currently there are four titles available in this series:

1. Enhanced Automated Immunomagnetic Separation (eAIMS) for *Escherichia coli* O157
2. Norwegian Institute for Food and Environmental Analysis response to an "*E. coli* Outbreak" (using **Dynabeads®** EPEC/VTEC O103)
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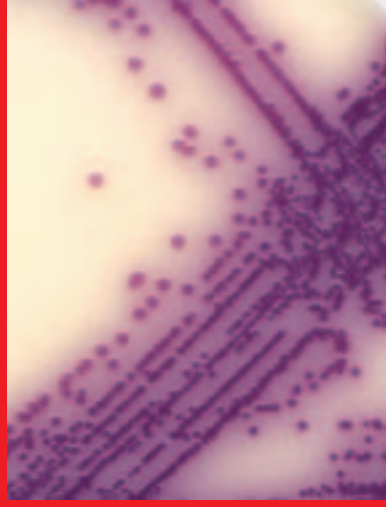

INNOVATION IN FOOD MICROBIOLOGY

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