Microbiologist

The magazine of the Society for Applied Microbiology ■ March 2014 ■ Vol 15 No 1 ISSN 1479-2699

Zoonoses

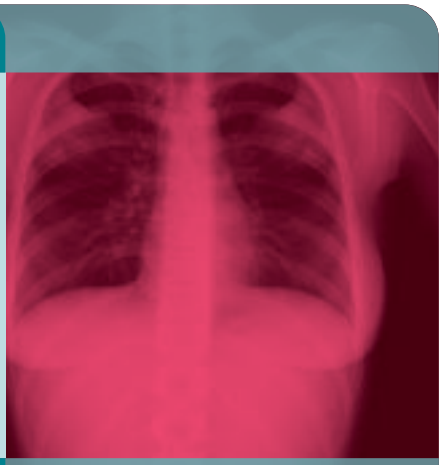
INSIDE

Wednesday 30 April 2014

Spring Meeting

8th broadening microbiology horizons in biomedical science meeting

● Control of infection: current status and future prospects



The Sheffield Hilton Hotel, Sheffield, UK

Programme*

*Please note that this is a provisional programme and likely to change. For the latest information please visit www.sfam.org.uk/en/events/index.cfm/springmeeting

09.25 – 10.25	**Tea, coffee, trade exhibition and registration**	
10.25 – 10.30	**Chairman's welcome**	
Chair:	**To be confirmed**	
10.30 – 11.00	**Antibiotic resistance and its implications for infection control**	Helena Parsons, Sheffield Teaching Hospitals, Sheffield, UK
11.00 – 11.30	**Viruses and their infection control implications**	Michael Ankcorn, Sheffield Teaching Hospitals, Sheffield, UK
11.30 – 12.00	**Challenges for the antimicrobial application of bacteriophage technologies**	Michael Mattey, Fixed Phage, Glasgow, UK
12.00 – 12.30	**Patient experience of surgical site infection**	Judith Tanner, De Montfort University, Leicester, UK
12.30 – 14.00	**Lunch and trade exhibition**	
Chair:	**To be confirmed**	
14.00 – 14.30	**How clean is my hospital?**	Stephanie Dancer, NHS Lanarkshire, UK
14.30 – 15.00	**Natural chemical diversity to combat infectious diseases**	Marcel Jaspars, University of Aberdeen, UK
15.00 – 15.30	**Natural antimicrobials, the future of infection control?**	Valerie Edwards-Jones, Manchester Metropolitan University, UK
15.30 – 16.00	**Infection control**	Martin Kiernan, Southport and Ormskirk Hospital NHS Trust, UK
16.00	**Close, tea and coffee**	

IBMS CPD ACCREDITATION 6 POINTS

To register online for this meeting please visit www.sfam.org.uk/spring
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Microbiologist the magazine of the Society for Applied Microbiology

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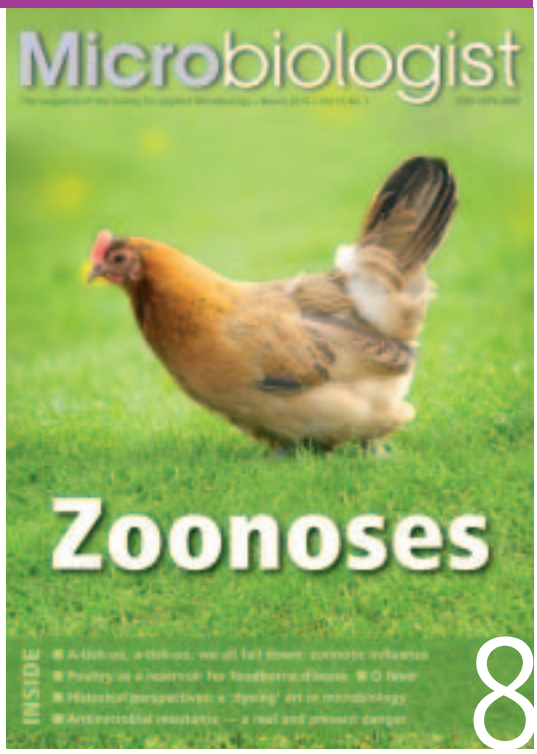

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editorial

Nancy Mendoza reviews the content of this issue of *Microbiologist*

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, Nancy Mendoza at: **nancy@sfam.org.uk**

Nancy Mendoza

Zoonoses

For many of us, the greatest fear of infectious disease comes from newly emerged pathogens that scientists and clinicians are unprepared to deal with. Consider also our globalized lives (see *Microbiologist* Vol. 14 No.3) and we observe the pressing need to understand the emergence of new infectious disease. We watch on as pathogens evolve, change, and recombine to challenge human health and well-being.

Some 70% or so of infectious diseases affecting humans have their origins in other species. The microorganisms responsible have been able to jump the species barrier, often overcoming evolved tropism and, in many cases, changing due to unusual circumstances. We might, therefore, reasonably expect that emerging human diseases will continue to arise from wild and domesticated animals.

Take MERS-CoV (the Middle East Respiratory Syndrome Coronavirus), for example (see *Microbiologist* Vol. 14 No.3). This newly emerged virus has now been found in camels and its close relative was identified in bats, which adds weight to the assumption that the human disease caused by MERS-CoV has its origins in a virus that infects animals.

All this is not to say, as Ian Jones points out (see page 8), that we should be afraid every time the cat sneezes, but it does highlight an important area of research — zoonoses.

Zoonoses are the focus of this year's S*f*AM Summer Conference (30 June to 3 July in Brighton, see page 36), which has been organized in conjunction with the Med-Vet-Net Association. In this edition of *Microbiologist* we explore perhaps the most famous zoonotic disease, with an article on influenza by Ian Jones (page 8); Frieda Jorgensen and Caroline Willis look at poultry as a reservoir for foodborne disease — *Campylobacter* and *Salmonella* infections, in particular (see page 13); and we are introduced to Q fever by Susannah Froude, Owen Seddon and Brendan Healy (see page 16).

Policy

Microbiology, like any scientific discipline, relies on a suitable political and economic environment. We need policies and laws that are enabling of high-quality science; and when there is an opportunity for microbiology to contribute solutions to the challenges facing society, we need Governments that are prepared to mobilize resources to our community.

When policy discussions are focused on broader areas of science, we often turn to the Society of Biology for collaboration and to provide a single strong voice for scientists working in biological sciences. As such, we have contributed to a number of Government and other consultations over the years. But sometimes the issues are of direct relevance to you, the applied microbiology community.

As an example, in 2013 the UK's political spotlight fell on the issue of antimicrobial resistance. We responded with written evidence to the House of Commons Science and Technology Select Committee (summarized, see page 22) and put forward our Executive Committee member, John Threlfall, as an oral witness. John and I attended the Select Committee on 18 December where John and three other experts answered questions from MPs.

As 'the voice of microbiology', there are times, like this, when we can contribute to policy discussions that have a direct impact on the ability to do microbiology for the benefit of society. We would love to hear from you if you are interested in being involved in this work. Email us at communications@sfam.org.uk.

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president's column

SfAM President, **Professor Martin Adams** reflects on the Society's continuing contribution to advancing the science of applied microbiology

Since its founding, the name of our Society has been changed twice, but for most of its history the word 'applied' has featured prominently. The significance of this should not be underestimated, nor should it be a diversion from the excellent, rigorous work our Members do in a broad field that includes academia, industry, and human and veterinary health services. It is a matter of fact that, as a subject, microbiology grew explicitly around the application and impact of microorganisms in the real world. Along the way it has, of course, been necessary for microbiologists to pursue more fundamental questions about what microbes are, in order to understand what they do, but it is generally recognized that this is not where the main impetus for development came from.

To quote Topley and Wilson's *Principles of Bacteriology, Virology and Immunity* (sixth edition): "*From Pasteur onwards the great majority of investigators have been more interested in what bacteria do than in what they are...*"

The many different ways in which microorganisms can influence our lives is well illustrated by the diversity of papers published in our journals and in the topics of our meetings. Just this year the SfAM year planner, which I trust occupies pride of place on your wall, contains meetings on biodefence, food contamination, activated sludge, control of infection and zoonoses. We obviously have a lot of areas to cover and cannot address them all in a single year, but I don't think we do too badly. Even if one of the current offerings doesn't correspond exactly with your immediate day-to-day interest, it is often worth making the effort of attending. I always maintain that I learn far more at meetings on topics in which I am not already well versed.

We also recognize a commitment to represent the world of applied microbiology to society at large — Government and regulatory authorities as well as the general public – to influence and inform policy, to increase public engagement and to inspire the next generation of microbiologists. In doing this, it is often more effective to speak with a bigger voice through collaboration with like-minded organizations as we have done, for example, with the Society for General Microbiology on recent position statements on Food Security and on Sexually Transmitted Infections.

Similarly, the Society of Biology (SoB) is an umbrella for a large number of learned societies, including ourselves, where we have many interests in common and where we can gain from acting together. This was the case with our contribution to the SoB's response to the call from the UK House of Commons Science and Technology Select Committee on the topic of antimicrobial resistance. The same issue does, however, illustrate the distinctive voice we still have when, as a result of evidence we submitted independently of the SoB response, John Threlfall, a Member of our Executive Committee, was invited to Westminster in December to give evidence to the Committee in person.

Beyond Government, it is important that we increase public awareness of applied microbiology more generally and provide expert commentary when it is in the news. This is important and worthwhile work but can have its pitfalls as well. The lure of fame can be seductive; I well remember how after one of my own brief appearances on television, I fully expected to be pursued with offers of a series or at the very least a small part in '*The Archers*'. Inexplicably, I was overlooked, a blow from which I am only just recovering.

Under the pressure of a live interview the urge to say something to fill the void can be unbearable. One wants to sound reasonably chatty, not too ponderous and measured, but it is equally important that misleading messages are not conveyed. I am reminded of the occasion in P. G. Wodehouse's '*Jeeves*' stories when Bertie Wooster at breakfast was warned that "*modern medical science held that a four-inch sausage contained as many germs as a dead rat*."

Reaching a younger audience is a particular challenge, requiring skills to both inform and entertain. Sometimes alas these can go a little out of balance. A colleague of mine working with young children and a carefully designed teaching booklet, encouraged the children to colour in images of foods most likely to harbour dangerous microbes. One young girl vigorously colouring in the picture of a piece of cake was asked "Is that dangerous?" to which she replied "*No, but I just like colouring in*."

Martin Adams
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

In the last issue of *Microbiologist* (Vol. 14 No.4, page 21), Dr Mark Downs, Chief Executive for the Society of Biology wrote a fascinating and interesting article gazing into a crystal ball to view where the Society of Biology would be in 20 years' time. The article had been prompted by Members of his Society asking this question. I thought it would be useful if I tried to offer a similar perspective for Members of the Society for Applied Microbiology (S*f*AM).

I think the first thing to say is that, at this moment in time, the S*f*AM Executive Committee (EC) don't have any plans in the short or medium term for a full merger with the Society of Biology, or any other Society for that matter. The EC is, however, keen to further relationships of co-operation and collaboration with like-minded organizations. And it is worth noting that the EC will continually review this position so as to keep pace with changes to the wider context in which the Society operates.

All that said, it is impossible to accurately predict what will happen in the next 20 years; like trying to predict the weather in the UK, the further out the forecast is, the more likely it is that it will be wrong. Where S*f*AM will be in the next 5, 10 or 20 years will be affected by many variables, which include social, economic, political and technological factors, amongst many others. The Society's current policy for working in close partnership, collaboration and co-operation with an umbrella Society such as the Society of Biology is very clear and unambiguous. Where possible, we will work closely in areas where there is a generic interest, including around relevant policy matters, and in education and public engagement. To this end, and to show further commitment, the EC of S*f*AM have recently agreed an increase in the amount of funding we provide to the Society of Biology to further enhance and facilitate this type of work.

Furthermore, I agree wholly with Mark's statement (*Microbiologist*, Vol. 14 No.4) that it is extremely important that a single and strong voice for biology enables a coherent message to be given to Government ministers and other major end users of scientific research, knowledge and skills; not just for biology, but also including chemistry, physics and mathematics. This promotes a collective argument for the value and contribution that science makes to society as a whole.

Mark's piece has sparked some of us to think back to the process that took place in the 1970s to form the Royal Society of Chemistry (RSC). We find that although there is some superficial similarity, comparison of an imagined amalgamation of the biology societies into a single Society is misleading. The formation of the RSC involved the bringing together of just four bodies — the Chemical Society, the Faraday Society, the Society for Analytical Chemistry and the Royal Institute of Chemistry. This was not without difficulties, but these pale into insignificance when compared with the potential problems associated with blending together more than 90 different life science societies into one single body.

Amalgamation of organizations can be very challenging, in particular when the organizations have long histories and disparate individual identities.

Another difficulty would be deciding what happens to the assets of each entity, including financial reserves. If one partner to any merger was financially not as strong as the others, then a merger will never be straightforward.

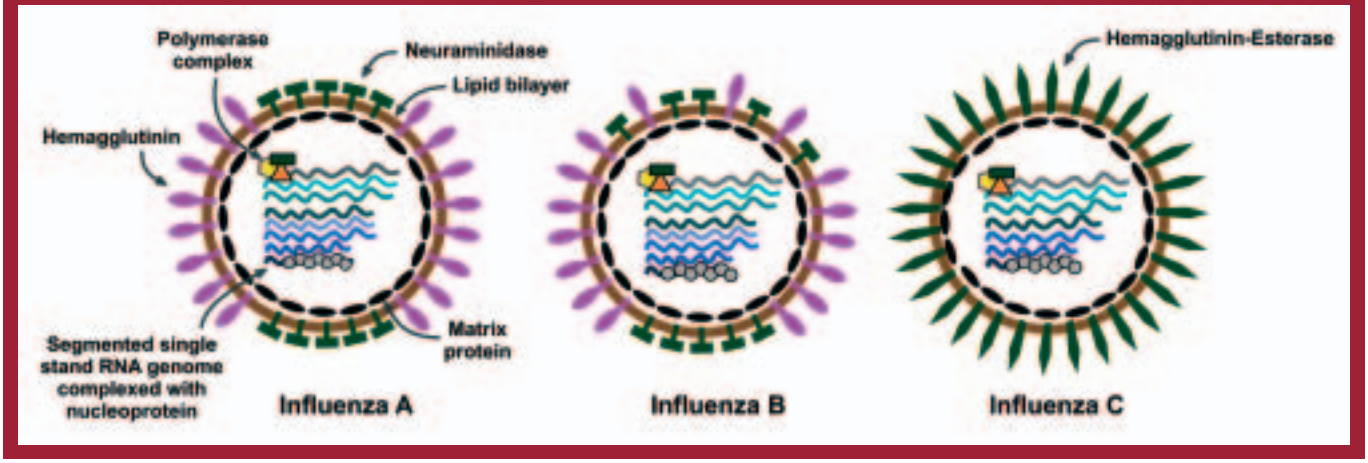
Any discussions between the organizations considering amalgamation of some kind must always be based on trust, honesty, respect and integrity by both parties. As well as these fundamental principles, it is important to consider the impact of any mismatch between cultures of organizations involved. In the case of S*f*AM, we are known for being the 'Friendly Society'; offering value for money to our Members; being modern, innovative and progressive in our approach; and for our inclusivity to all Members. It would be challenging to achieve a satisfactory alignment with another organization that does not, or will not, share these values.

As the President and I pointed out in our joint column in the last *Microbiologist*, S*f*AM is in a very healthy position when a number of key indicators of success are measured. The Society has been in existence, under its various names, for 83 years and as such has a strong and distinct history. I do hope that for the next 83 years we will continue to provide services to our Members that are value for money, and that we continue to benefit the wider general public as efficiently and effectively as possible.

Philip Wheat
Chief Executive Officer

A-tish-oo, a-tish-oo, we all fall down: zoonotic influenza

Figure 1. Relatedness of influenza viruses
The relatedness of influenza viruses is obvious from their appearance: only influenza C varies to any degree with one less genome segment and a different surface protein. Biologically however, they vary in clinical significance and pandemic potential with only influenza A considered an active threat



That infectious disease spreads from animal to man is not new. Many, perhaps 70%, of our established infections have their origins in other species (Chan *et al.*, 2013) and all forms of microorganism, bacteria, parasite, fungi and viruses cross the species barrier at irregular intervals or under unusual circumstances. The surprise perhaps is that it is not more common: when the cat sneezes we generally do not get infected!

The explanation for this species preference, or tropism, is the co-evolution of host and pathogen over time that favours the survival of both. Put simply, a microorganism gives up some of its virulence and generally increases its transmissibility by becoming ever more dependent on host functions, and those functions become so specific that even a closely related species cannot provide them.

Jenner's original use of cowpox as a vaccine for smallpox exemplifies the point. The viruses were closely enough related such that immunity to one provided protection against the other, yet cowpox was so adapted to its various origins (Carroll *et al.*, 2011) that it represented no infection risk in man, it replicated only in isolated pocks and did not spread. The property of limited infection and a failure to spread, but coupled with the fear that it might, is the core concern behind the virus zoonosis that is ever with us, influenza.

The current discussion around influenza concerns almost exclusively influenza A strains, those strains that have historically given rise to influenza pandemics, but there are three types of influenza in all, A, B and C (Figure 1).

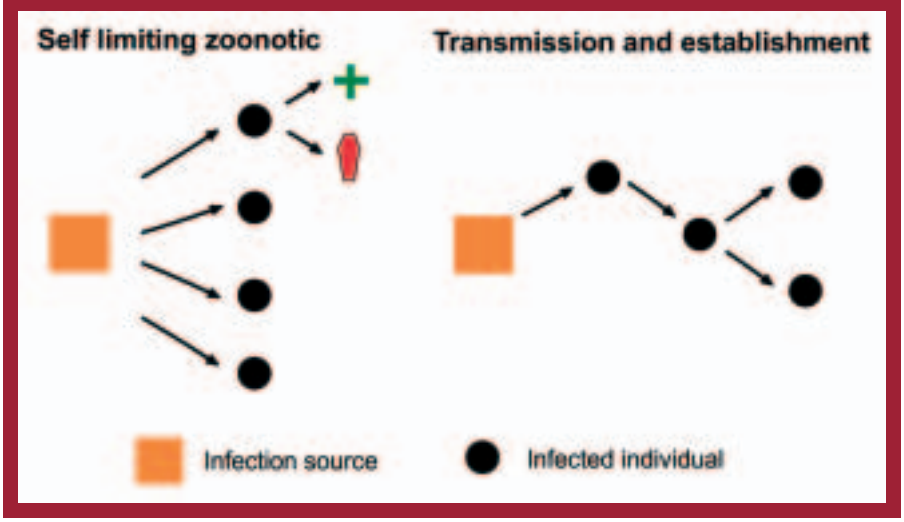
Influenza C is found almost exclusively in man (occasionally in swine), influenza B is found in humans and marine mammals, and influenza A infects many species including humans, swine, equines, bats and, predominantly, avians.

The restricted tropism of influenza C and B viruses is matched by the clinical severity of the respiratory diseases they cause. Influenza C infection is invariably mild (Kauppila *et al.*, 2013) and while influenza B infection can give a clinically notifiable bout of flu, it is not as severe as that typified by influenza A infection (Labella & Merel, 2013). Neither virus gives rise to pandemics.

These clinical features reflect the time the requisite viruses have had to adapt to their human hosts, influenza C ~8,000 years, influenza B ~4,000 years, but influenza A perhaps only 100–200 years (Suzuki & Nei, 2002; Xu *et al.*, 2011).

Evidently, influenza B and C made the jump from an animal reservoir early and have now reached a form of evolutionary stasis, where the shuffling of geographic isolates and the accumulation of a sufficient number of susceptible individuals in a population controls the level of infection (Yang *et al.*, 2012).

Figure 2. The pattern of virus zoonosis versus virus spread
Left: direct infection of man leads to death or resolution with immunity, both dead-ends for the virus which then becomes extinct.
Right: only if adaptation occurs is spread possible among the new population. So far H5 and H7 avian influenza outbreaks have followed the pattern on the left



Influenza A by contrast has split into many subvariants identified by the sequence of the major surface proteins of the virus, the haemagglutinin (HA/H) and to a lesser degree the neuraminidase (NA/N) (Xu *et al.*, 2012). There are currently 18 H types and 9 N types, some variants of which, those that have given the pandemics of the past, have now adapted to man and begun the passage towards attenuation. They give us the seasonal influenza outbreaks, which since 1918 have involved only three variants (H1−H3), while other HA types occur predominantly in other species, particularly avian species, and infect man only rarely.

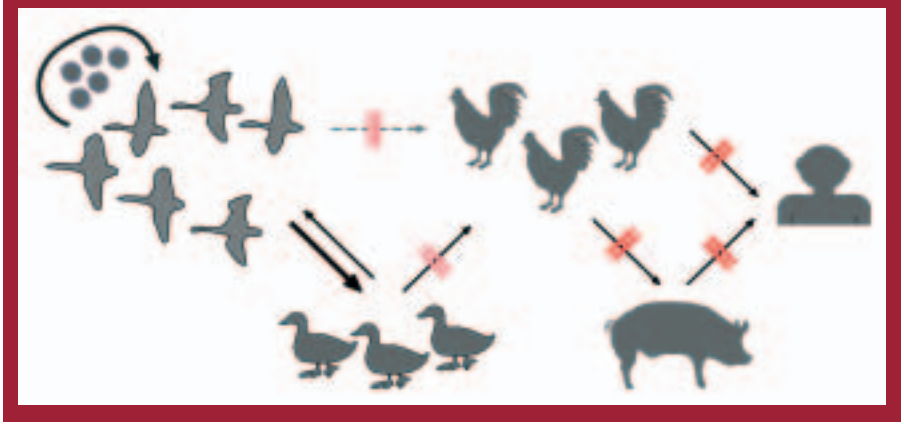
These latter A type viruses represent a zoonotic risk for two reasons. First, as they have not yet adapted to human cells, the viruses are not attenuated and their pathogenicity is generally high. Second, their presence in man allows the possibility of adaptation to allow transmission among individuals, which, as the virus is new and not susceptible to any pre-existing immunity, would lead to a pandemic. It is not necessarily true that a pandemic virus would also be highly pathogenic, but it is a possibility and the combination of a newly emerged highly pathogenic virus with efficient transmission is the worst case scenario for influenza risk assessment.

How do these occasional outbreaks occur and what, if anything, can be done to minimize them?

The ability of a zoonotic influenza type A to infect man depends on two parameters, the ability of the virus to enter and replicate in human cells, and the opportunity to do so. The latter explains why avian influenza outbreaks in farmed poultry are so unwelcome. If a suspect virus circulated in only, say, arctic terns, the opportunity to infect man would be very limited. However, if the same suspect virus finds its way into a broiler house of 10,000 birds it can be substantially amplified in a short time and has the opportunity of contact with man, directly via the handlers or indirectly via the food processing chain; both the H5 and H7 outbreaks that have triggered global alerts in the past 15 years, or so, originated in this way.

The H5N1 virus that came to prominence in Hong Kong in 1997, and has since spread across Asia, originated by 'normal' influenza evolution in migratory birds but became a threat when it infected domestic poultry destined for live poultry markets (Duan *et al.*, 2007).

Figure 3. The natural history of influenza A
This figure shows how outbreaks are so difficult to predict. Natural evolution among migratory birds finds its way to water fowl and then to local domestic poultry. Either directly or via intermediate domestic animals the virus then contacts man. Biosecurity possibilities are indicated but are only partly realistic



Similarly, the recent H7 outbreak in eastern China followed a mixing of avian influenza viruses circulating in Chinese and Korean birds around Shanghai in 2013 (Wang *et al.*, 2013). Infection of people was again associated with live poultry markets (Chen *et al.*, 2013) and their subsequent closure in several Chinese cities effectively curtailed the outbreak (Yu *et al.*, 2013).

Both the H5 and H7 viruses concerned were high pathogenicity viruses, that is, they cause a high mortality in some avian types and that pathogenicity is maintained when people are infected: nearly 60% of the global H5 human infections to date have been fatal, while the figure for the H7 outbreak is 30%. Importantly however, the viruses concerned did not adapt and there was no significant onward transmission (Figure 2).

Not all H5 and H7 strains are of high pathogenicity and neither are they the only strains implicated as potential human infections. H9 influenza is considered a threat (Capua & Munoz, 2013) and recently a Taiwanese woman was diagnosed with an influenza H6 virus (Shi *et al.*, 2013).

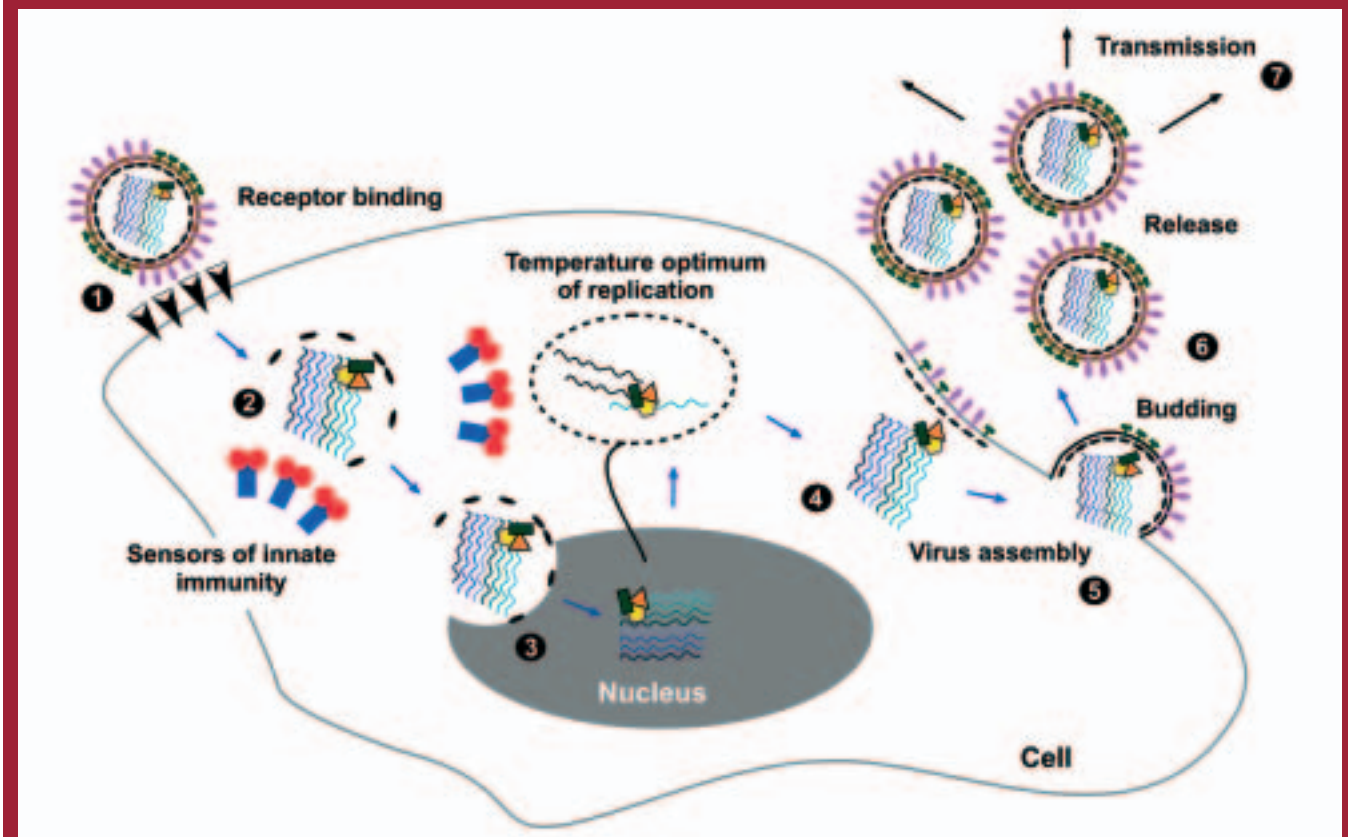
Regardless of the strain, the route to human infection is invariably via birds raised for human consumption and appropriate biosecurity measures can minimize the risk (Figure 3).

The factors that determine whether a suspect influenza strain can infect man when contact occurs are more varied. Barriers to infection exist from the very first steps of entry, binding between virus and the host cell receptor, to the very last, virus release and transmission (Figure 4).

Influenza viruses use a sugar molecule, sialic acid, as their receptor. A pocket at the tip of the virus haemagglutinin protein binds it, effectively trapping the virus to the cell surface. However, sialic acids typical of the avian gut (where avian influenza replicates) are different from those typical of the mammalian upper respiratory tract (where mammalian influenza replicates). A first barrier to infection then, is the ability of an avian influenza virus to attach to mammalian sialic acids (in turn a property of the haemagglutinin protein) particularly the amino acids that line the sialic acid binding pocket. In the main, avian viruses bind mammalian sialic acids poorly, although certain mutations can improve their binding, and this is one of the factors that has prevented the H5 and H7 viruses infecting man on a wider scale (Ayora-Talavera *et al.*, 2009; Xiong *et al.*, 2013).

If cell entry does occur, replication of the virus should follow. However, the capacity of avian and mammalian cells to replicate the same influenza strains differs, partly because the temperature optimum for the virus polymerase proteins varies. Avian viruses have evolved to work best at avian body temperatures (~39°C) whereas the mammalian upper airway is distinctly cooler (~33°C). Accordingly, avian virus polymerases are poorly efficient in human airway cells although this can be

Figure 4. Barriers to zoonotic influenza at stages in the replication cycle
Influenza replicates following a structured series of events from receptor binding (1), uncoating and intracellular transport (2), replication in the nucleus (3), production of virus components (4), assembly (5) and release (6). The final stage, transmission (7) requires completion of stages 1–6 and the availability of susceptible hosts



compensated for by a single amino acid change in a virus polymerase protein (Min *et al.*, 2013).

A further limit to virus replication is the effective closing down of the infected cell by an immune system alerted by virus infection. Triggering occurs at the level of the virus RNA, the product of virus replication, which is recognized as foreign by sensors of the innate immune system. Part of the adaptation of influenza to its host over time is the moderation of this response so that the virus remains undetected and a single virus protein produced in infected cells is the major effector involved. For an avian virus to succeed it must moderate the human sensors as does a human influenza strain if it is to avoid detection. Avian viruses can acquire mutations that allow this but in their absence, which is the case for most circulating avian viruses, the virus is stopped from spreading to any significant degree (Fan *et al.*, 2013).

A final restriction that any zoonotic virus must overcome, if it is to succeed in colonizing a new species, is transmission. If transmission fails then even the successful infection of an individual goes no further and the virus will become extinct. That transmission is not just the consequence of successful virus replication is demonstrated by the fact that zoonotic avian influenza has failed to establish in the human population despite infecting (and killing) many individuals.

The requirements for avian H5 influenza transmission among mammals were established recently in a series of now notorious experiments (Herfst *et al.*, 2012; Imai *et al.*, 2012). Unsurprisingly, mutations previously shown to switch receptor specificity were among those selected, but other mutations in the HA and polymerase genes were also required. No one configuration of mutations proved effective and, in an unexpected twist, H5 viruses adapted to transmission no longer killed the animal model in use. At least in these experiments, adaptation of avian H5 influenza to a mammal transmissible strain was at the cost of virulence, a relatively encouraging result for the risk assessment of avian H5N1 although it should be stressed that the experimental system used was not the same as the infection of man.

The influenza viruses present in avians and other species have been the source of pandemic influenza in the past and will be so again. However, the barriers a virus has to overcome to adapt to mammalian replication and transmission are considerable, and as the mutations required become characterized surveillance programmes can incorporate them into their analyses to provide early warning of strains that have at least part of the potential to cross the species barrier. In the longer term, vaccines capable of a degree of cross protection may provide some level of immunity against potential new pandemic viruses. In the meantime, monitoring and active biosecurity measures should minimize the potential for outbreaks even if absolute prevention is unrealistic.

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Poultry as a reservoir for foodborne disease

Introduction

Poultry and poultry products are recognized as the most significant source of human *Campylobacter* and *Salmonella* infections in the developed world, including the UK. Outbreak investigations and case-control studies investigating risk factors and transmission routes have identified poultry meat and eggs as major sources of infection. However, non-foodborne routes such as animal contact, and occupational or recreational exposure, are also important.

Poultry meat, and chicken liver or duck liver products were implicated as the source in 62 of 103 *Campylobacter* outbreaks reported to the Health Protection Agency (HPA) between 2000 and 2012. Eggs and poultry meat were implicated in 52 and 43 *Salmonella* outbreaks (of 382 in total reported to the HPA), respectively, over the same time period. In the EU, eggs and egg products were one of the main food vehicles associated with foodborne outbreaks, while broiler meat was the fifth most frequent cause of foodborne *Salmonella* outbreaks in 2008 (EFSA, 2010a). Moreover, data from the European Commission's Rapid Alert System for Food and Feed (2008) indicated that reports of microbiological contamination in poultry meat were more common than for any other food type. In an EU survey from 2008, raw chicken meat was frequently contaminated with campylobacters (approximately 80% of samples) but less so with salmonellas (approximately 16%) (EFSA, 2010b).

The extent to which different infection risk factors are associated with different sources can be inferred by combining case-control studies with source attribution studies (i.e., studies that determine the predisposition of specific genotypes to infect particular animals). Such studies have provided further evidence that poultry is the major source of campylobacteriosis.

Chicken Production and Slaughter

The introduction of microbiological criteria in legislation and EU targets for reducing *Salmonella* in broiler flocks has contributed to a decline in flock prevalence of this organism in some member states (EFSA, 2013), with only 3.2% of broiler flocks being positive for *Salmonella* in 2011. The UK *Salmonella* National Control Programme (NCP) has particularly focused on two serovars (*Salm.* Enteritidis and *Salm.* Typhimurium), but this has been accompanied by a general reduction in salmonellosis in the UK (Figure 1). *Salmonella* is now very rarely detected in UK broiler flocks. Only four broiler flocks in 2012 tested positive for *Salm.* Typhimurium out of a total of approximately 38,000 flocks tested, and none were positive for *Salm.* Enteritidis. There is no similar specific legislation for campylobacters in raw chicken meat, but authorities and industry in EU countries have introduced measures aimed at reducing *Campylobacter* contamination from farm to fork. However, in the UK the large majority of chicken flocks have tested positive at slaughter for campylobacters in recent years despite the introduction of stricter biosecurity measures.

Chicken flocks are usually colonized with *Campylobacter* as a result of environmental exposure but may show no signs of ill health when colonized, although bird health indicators have

Figure 1. Reports of non-typhoidal human Salmonella cases in the UK, 1993–2012

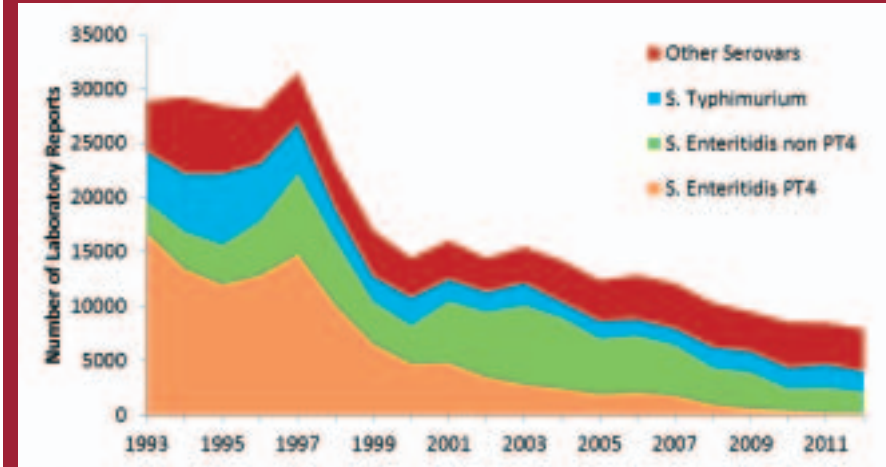


Figure 1: Reports of non-typhoidal human *Salmonella* cases in the UK, 1993– 2012

been associated with the likelihood of a flock being colonized. In most European countries, including the UK, chicken flocks are more likely to be colonized in the summer months possibly due to increased infection risks (Lawes *et al.*, 2012). Although poultry that are farmed outdoors may appear to be more at risk of becoming exposed and colonized there is no evidence that *Salmonella* is more prevalent in poultry farmed outdoors compared with housed birds. In contrast, campylobacters appear to be more prevalent in poultry farmed with access to range compared with housed birds.

Slaughter, plucking and evisceration, in particular, lead to carcass contamination and a significant proportion of carcasses will have high levels of campylobacters when a positive flock is slaughtered. In comparison, slaughter of cattle, pigs and sheep/lamb is associated with much lower levels of carcass contamination despite these animals also demonstrating high colonization rates (EFSA, 2013), probably due to a slaughter process that limits cross-contamination from gut material to the carcass.

Compared with the numbers found on poultry meat and liver surfaces, bacterial numbers inside tissues are low but nonetheless may be significant when undercooking occurs. *Campylobacter* can be present inside liver and meat tissue, although most infections are thought to relate to *Campylobacter* cells on the surface. In one study, 30 chicken livers were examined with campylobacters being detected in 27 and 2 had internal counts of more than 1,100 CFU/100g. An increased number of *Campylobacter* outbreaks have been observed in recent years, and many of these have been linked to the consumption of chicken or duck liver pâtés and parfaits where the livers had been inadequately cooked. Campylobacters can also be present inside chicken breast fillets (approximately 20%) but at very low levels (an average of 15 cells/100g in positive fillets has been reported).

Until the late 1990s, it was common practice to add antibiotics to animal feed, including poultry feed, in order to promote growth of the animals. This practice selectively encourages the growth of antibiotic-resistant bacteria. Thus, the use of enrofloxacin as a growth promoter in poultry has been linked with an increase in ciprofloxacin resistance in campylobacters and salmonellas, whilst the use of avoparcin correlated with an increase in the prevalence of vancomycin-resistant enterococci in chicken meat (Willis, 2000). While a ban on the use of medically important antibiotics as growth promoters was introduced in the EU in 1999, this practice has continued in other parts of the world. Moreover, some studies suggest that the decrease in the use of growth promoters has been accompanied by an increase in the use of prophylactic and therapeutic antibiotics. Therefore, there is a continued risk of pathogenic bacteria in poultry developing antibiotic resistance patterns that may make infections with these organisms very difficult to treat effectively in humans.

Egg Production

Raw shell eggs can be contaminated with *Salmonella*, either presenting as shell contamination due to contact with faeces after laying (migration of the organism through the shell is possible) or as egg content contamination due to colonization of the hen's oviduct. *Salmonella* Enteritidis, in particular, is known to be closely associated with eggs. In the late 1980s, the incidence of *Salm*. Enteritidis Phage Type (PT) 4 infections increased sharply in the UK and Europe, and this increase was linked with consumption of poultry meat and shell eggs. Studies have indicated that *Salm*. Enteritidis has a greater ability to persist in the laying hen reproductive tissues and egg contents, compared with other *Salmonella* types (Van Immerseel, 2010). In 1998, the Lion Quality Scheme was introduced in the UK, setting down a code of practice covering all stages of egg production, and including vaccination of pullets destined for egg-producing flocks against *Salm*. Enteritidis. Between 1997 and 2000, there was a 54% reduction in *Salm*. Enteritidis PT4 cases reported in the UK, and this decline has been maintained since then (Figure 1). *Salmonella* contamination levels in British eggs are now extremely low, with an FSA study in 2003 finding *Salmonella* in only 0.34% of eggs tested. However, egg-related outbreaks of *Salmonella* still occur, and these are largely linked with catering eggs produced outside the UK. For example, in 2011, 262 people in the UK were infected with *Salm*. Enteriditis PT14b. The source of infection was traced to eggs from a specific shed on one farm in Spain, which supplied implicated catering establishments in the UK. It is important to ascertain the proportion of foodborne disease which is linked to imported foods so that the effect of national intervention measures can be assessed.

These figures reflect that only one laying flock tested positive for *Salm*. Enteritidis and two for *Salm*. Typhimurium out of 4,042 flocks included in the UK NCP in 2012. However, across the EU 4.2% of laying hen flocks were positive for *Salmonella* spp. in 2011 causing concerns for imported eggs (EFSA, 2013).

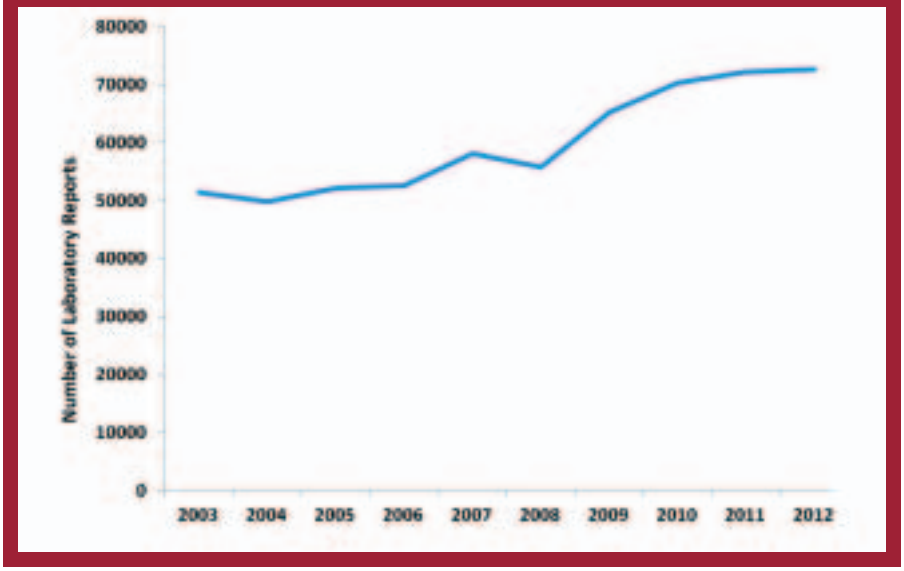
Environmental Transmission

It has been demonstrated that, when raw chicken is handled and prepared in a domestic kitchen, contamination of hands, kitchen surfaces, cloths and equipment with *Salmonella* and/or *Campylobacter* frequently occurs (Gorman *et al.*, 2002). Moreover, when contaminated eggs are whisked, aerosols may spread over 40cm away from the mixing point. Contamination was not reliably removed during a laboratory simulation of a typical washing-up process (Mattick *et al.*, 2003). Therefore, there is a considerable risk of cross-contamination from raw chicken and eggs to other ready-to-eat foods that are handled in the kitchen. Of 101 outbreaks in private houses, 44 were associated with poultry and cross-contamination was described as a contributory factor in 28 of these (Ryan *et al.*, 1996).

Future Strategies

In the EU, legislation has contributed to lowering the *Salmonella* presence in poultry, but some countries still have far to go. The current strategies to further reduce salmonellosis arising from the poultry reservoir require continued efforts on improved egg and broiler meat hygiene supported by vaccination. The current UK Food Standards Agency target for *Campylobacter* is to reduce the proportion of chicken carcasses with more than 1,000 CFU/g of neck-skin to 10% by 2015 (in 2008, 27% of samples had > 1,000 CFU/g). While control measures applied at the chicken meat processing stage will have no impact on environmental transmission arising from the farm reservoir, interventions such as rapid crust chilling of carcasses can result in significant reductions of the levels of campylobacters on carcasses. The Scandinavian countries have a particularly good record of low pathogen prevalence in poultry, and while differences in climate and livestock density may explain this to an extent, industry practices including all-in-all-out systems within farms and other high-level biosecurity measures also play significant roles. Promising new interventions including fatty fish-based feed additives and novel *Campylobacter* vaccines exploiting knowledge of glycosylation may help reduce the presence of campylobacters in poultry in the future.

Figure 2. Reports of human Campylobacter cases in the UK, 2003–2012



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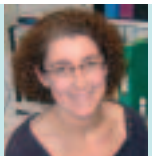
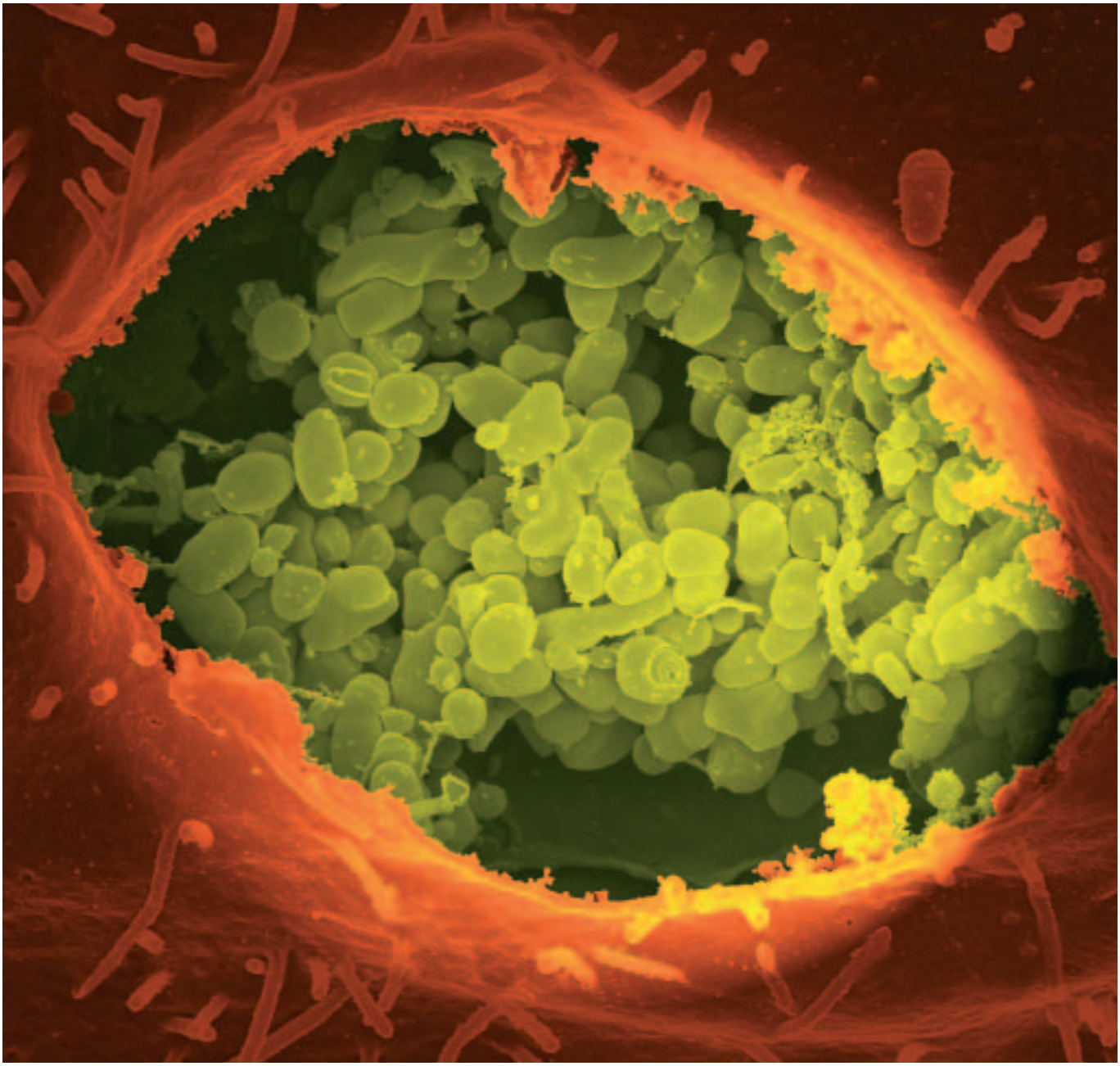


Frieda Jorgensen (left) **and Caroline Willis** (right)
Public Health England Food Water and Environmental Microbiology Services



Q fever

Q fever is a worldwide zoonotic disease, caused by the rickettsia *Coxiella burnetii*. It was first described in Australia in 1937 following investigations into a febrile illness outbreak in abattoir workers. 'Q' stands for 'query' as the cause of the illness was not known.

C. burnetii is an obligate intracellular Gram-negative organism. It can be cultured in embryonated eggs or cell cultures but not axenic media. Intracellularly it survives within the acidophilic phagolysosomes and has a doubling time of 20 hours. It can exist in spore-like forms that are able to survive in the environment, resisting heat, desiccation and disinfection. Spores have been detected in areas long after infected animals have left (Maurin & Raoult, 1999).

Because culture of *C. burnetii* is difficult, lacks sensitivity, and requires level 3 biosafety facilities, diagnosis is usually based on serology. PCR on blood, urine or tissues can also be used for diagnosis in certain specific circumstances.

C. burnetii is found throughout the animal kingdom and is most commonly transmitted to humans via domestic animals. The primary reservoir is cattle, goats and sheep with 21% of

UK dairy herds infected (HPA, 2014). Animals are usually asymptomatic but shed *C. burnetii* in urine, faeces and during parturition.

The infectious dose is thought to be as little as a single organism. Inhalation is the commonest means of acquisition of infection through contact with infected animal fluids or fomites in the environment. Outbreaks have been associated with windborne spread over large areas (including urban areas) (Wallensten *et al.*, 2010). The largest outbreak of Q fever reported occurred in the Netherlands, where since 2007 there have been over 4,000 cases related to intensive goat farming in the proximity of densely populated areas. Rarer modes of reported transmission include ingestion (e.g., unpasteurized milk), human to human (reported in an obstetrician performing a termination of pregnancy on an infected patient), laboratory acquired infections, and in one case a blood transfusion (Anonymous, 1977). It is considered as a potential bioterrorist agent (Category B) (Madariga, 2003). Q fever is considered an occupational hazard for farmers, vets and abattoir workers.

Acute Q fever

50 to 100 cases of acute Q fever are diagnosed and reported annually in the United Kingdom. The true incidence is likely to be higher as 60% of infections are asymptomatic. The incubation period is between 5 and 40 days depending on the size of the infecting dose. Presentation is variable due to host factors, the extent of the exposure and bacterial virulence factors. A diagnosis of Q fever should be considered in all febrile patients with a history of exposure to domestic ruminants or raw milk, and in patients with any of the clinical symptoms or syndromes detailed below.

The main features of acute Q fever are 'flu-like' symptoms, pneumonia and hepatitis; although the presentation is highly variable and these three symptoms may co-exist. A typical illness would be fever, chills and a headache that spontaneously resolve over two or three weeks. Classically, the temperature is high (39 to 40 degrees) and lasts for 5 to 14 days without diurnal variation. Headache is often a prominent feature, even in cases of pneumonia.

Q fever pneumonia accounts for less than 5% of UK community acquired pneumoniae and is rarely fatal. Q fever hepatitis presents with fever, abdominal pain and elevated liver enzymes. Hepatomegaly may be present, but jaundice is rare. 'Doughnut' granulomas (granulomas with a central clear space and a fibrin ring) are the typical histological feature, but these are not pathognomonic for Q fever.

A transient bacteraemia occurs during initial infection and haematogenous spread to other organs can result in life-threatening complications of meningoencephalitis or myocarditis. Around 2% of symptomatic cases require hospitalization. Antibodies become detectable two to three weeks after the onset of disease. *C. burnetii* phase II antibodies are produced in acute disease while phase I antibodies are seen in chronic disease. Serological tests available include complement fixation test (CFT), ELISA and microimmunofluorescence. These tests have different performance characteristics that are beyond the scope of this article; however, microimmunofluorescence is considered the gold standard test. Acute Q fever is treated with 7 to 14 days of doxycycline with continuation for three days after fever resolution. Treatment is most effective if given within three days of the onset of illness.

Table 1. Proposed criteria for the diagnosis of chronic Q fever from the literature

Phase 1 IgG > Phase 2 IgG, Elevated Phase 1 IgG or Phase 1 IgA
Phase 1 IgA > 320 and CFT > 128
Phase 1 IgG > 800, Phase 1 IgA not contributory
Phase 1 IgG > Phase 2 IgG + Phase 1 IgA > Phase 2 IgA
Phase 1 > Phase 2
High Phase 1 IgG and Phase 1 IgA

Chronic Q fever

Chronic Q fever is estimated to occur in between 1–5% of infections with *C. burnetii.* There have been many proposals for serological diagnosis of chronic Q fever (Table 1). A phase I IgG > 800 in the correct clinical context is suggestive of chronic infection. The Dutch cohort has also recently proposed a definition for chronic Q fever (Table 1) (Wegdam-Blans *et al.*, 2012). There is, however, no perfect serological cut off for the diagnosis of Q fever, not least because microimmunofluorescence serological results from different laboratories are not directly comparable.

Endocarditis is the principle clinical manifestation, accounting for 60–70% of cases of chronic Q fever, and making up around 3% of all cases of endocarditis (Raoult *et al.*, 2000). Other manifestations include vascular infections, osteoarticular infections, chronic hepatitis and chronic lung infections. Factors associated with chronicity include increasing age, immune suppression (including post-transplant, malignancy, chronic renal failure and HIV). Chronic Q fever has a slow progression but untreated infection can be fatal.

Q fever endocarditis is an indolent infection with a non-specific presentation, particularly in the early stages where typical manifestations are low-grade fever, fatigue and weight loss. With more advanced disease more typical features may develop, including valvular incompetence and cardiac failure. Diagnosis can be difficult because of the non-specific presentation and because vegetations are often too small to be detected by echocardiogram. Although Q fever endocarditis has a low mortality, the most appropriate treatment duration is uncertain and there is a significant risk of relapse when antibiotic treatment is withdrawn.

Vascular infections account for around 8% of chronic Q fever cases and tend to co-exist with underlying vascular abnormalities, including aneurysms and prosthetic vascular grafts. It presents with systemic features of infection, weight loss and abdominal pain. More catastrophic presentations of aortoenteric fistulae, aneurysmal rupture and lower limb embolization have also been reported. The overall mortality in case series is around 24% and relapse rates are high. Surgical resection and long-term antibiotic therapy are the two most established modalities of treatment.

Osteoarticular disease is probably under-reported. It should particularly be suspected if histology shows tuberculoid appearances without the isolation of a mycobacterial pathogen. Spinal osteomyelitis may also exist contiguously with an aortic graft infection.

Chronic hepatitis may occur in conjunction with endocarditis, or as a separate pathology. The difficulty in establishing endocarditis by imaging alone should prompt a thorough investigation to exclude concurrent valvular pathology in all cases of hepatitis. Chronic lung involvement, although apparently rare, may be implicated in some cases of pulmonary fibrosis or pseudo tumours.

Q fever infection during pregnancy exists in both acute and chronic forms. Acute Q fever most commonly leads to placentitis, with secondary complications due to immune-complex formation (vasculitis, thrombosis, placental insufficiency). In some (but not all) studies, infection has been associated with premature birth and spontaneous abortions (Carcopino *et al.*, 2009).

Surgery alone does not eradicate disease but has been shown to reduce morbidity and mortality in case series for vascular infections, and may be necessary for haemodynamic instability in endocarditis. It must be combined with prolonged antibiotic administration. The current standard is a combination of doxycycline and hydroxychloroquine, which is believed to act synergistically by increasing the phagolysosomal pH.

Other agents with activity against Q fever include rifampicin, quinolones and clindamyin, though there is a smaller evidence base for these agents. The optimum duration of antibiotic therapy has yet to be determined and recommendations range from one year to lifelong administration, with serological monitoring of phase I antibody titres. Proposed criteria for adequate response include a four-fold decline in phase I IgG titres, or a reduction to less than 1:200. The value of serology in determining outcome of treatment has, however, been questioned.

The Dutch Experience

Between 2007 and 2011, the Netherlands experienced the largest outbreak of Q fever yet recorded, with over 4,000 cases of acute Q fever notified. The first indicator of the epidemic was an increase in the numbers of atypical pneumonias being notified, followed by an increase in Q fever notifications. Outbreaks continued to occur, eventually in 10 separate clusters around the same region, with adjacent geographical spread, over 2008 and 2009 (Roest *et al.*, 2011).

Epidemiological investigation suggested that infection was most probably related to goat farming and airborne spread, partly facilitated in 2007 by the unusually hot and dry weather. Various measures were taken to gain source control of the epidemic, including stringent farm hygiene and vaccination of infected herds, followed by the culling of pregnant animals at Q fever positive farms.

It is not clear why the Netherlands experienced such an outbreak when other countries have similar background factors (a high seroprevalence in cattle in close proximity to the human population). An expansion of farms and an increase in goat density were two likely factors, as well as an increasing population density in the area. There may also have been pathogen-specific factors, perhaps due to a particularly virulent strain of *C. burnetii*. The outbreak has given the opportunity for research into acute and chronic Q fever in a large cohort, something that is not normally possible because of the low incidence of clinically recognized infection.

Conclusion

Q fever is a worldwide zoonosis, and should be considered in the patient with characteristic symptoms of fever, 'flu-like' illness, pneumonia or hepatitis and a relevant exposure history. The commonest manifestations of chronic Q fever are endocarditis and vascular infection, and chronic Q fever should be suspected in patients with unexplained fever, negative blood culture and underlying vascular or cardiac disease.

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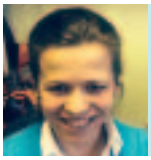
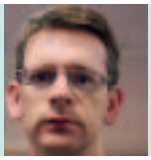




Susannah Froude (left), **Owen Seddon** (centre) **and Brendan Healy** (right)

historical**Perspectives**

Figure 1. *The famous Lion's Gate in Delft*

Figures 2 and 3. *A representation of the first microscope (left) and a compound microscope (right)*

A 'Dyeing' art in Microbiology

In the scientific community, methodologies, practices, techniques and systems continue to change, evolve and develop. Throughout all of this there are often constants — spare a thought for the simple **stain**; a foundation in diagnostic cellular identification.

There are approximately 4,500 published formulations for stains and countless variations, not least due to personal changes and even mistakes. Staining methods have long been used as an essential aid in the diagnosis of disease, cellular differentiation and bacterial identification, and still have an irreplaceable role in many diagnostic pathology departments. Stains will be found in routine use in cytology, histology, haematology, microbiology and parasitology, for studying a range of specimens in both *in vitro* and *in vivo* conditions.

As we now move further into the molecular and electronic age, will we always need the stain? Will multinational corporations fade away? Will the microscope only be seen in museums? Will a biomedical scientist's skills be even further eroded and devalued? Anyone can push a button and call a helpline. Only skilled biomedical scientists can interpret a stain and use the technique.

Where did it all begin?

Ask a tourist what Delft is famous for, and they will more than likely answer 'delicate blue collectable pottery', rather than microscopes and the origins of staining techniques, but they'd be missing something.

Some of the most important origins of staining date back not to a scientist, but to a Dutch draper known as Antoni van Leeuwenhoek (1632–1723), whose hobby was the grinding of glass which he used to examine threads in fabrics and later a variety of biological samples. Born Thonis Philipszoon, he became known as van Leeuwenhoek perhaps as he was born in a house on the corner of Lion's Gate in Delft, van Leeuwenhoek translating literally as *from Lion's Corner* (Figure1).

Due to his skills in grinding glass lenses, which at the time were no more than slivers of glass, van Leeuwenhoek is today credited with the discovery of the microscope, and was the first to observe blood cells and bacteria using this simple invention. He was the only one who made these observations and it would take many years before other people could confirm them; Figure 2 shows a representation of this first microscope, a far cry from the advanced systems available today.

The 17th and 18th centuries saw many advances in science, and competition between scientists was fierce as they strived for discoveries and acknowledgement. van Leeuwenhoek avoided confrontation and carried on his work quietly. Had it not been for a learned friend, Dr Regnerus de Graaf, he may never have been credited with his discovery, it being de Graaf who sent the first transcripts of his work to the Royal Society of London. Founded in 1660, the Royal Society, possibly the oldest scientific society, had many now legendary scientists and inventors as founder members including Robert Boyle, Christopher Wren, Isaac Newton and Robert Hooke.

It was Robert Hooke who furthered the development of the microscope and is one of a number of scientists credited with the invention of the compound microscope (Figure 3), using more than one lens and allowing magnification up to x1,000 for the first time.

Once we had microscopes, we needed colours to differentiate cells. Working alone in Delft in 1714, van Leeuwenhoek was examining the muscles of fat and lean cows, and found that the material was too transparent for clear observation. It is here that his work on the theory of

Table 1. Staining timeline

Date	Event
1590–1610	Initial records of the microscope with Hans and Zacharias Janssen
1652–1720	Development of the van Leeuwenhoek microscope and first staining techniques with saffron
1660	The Royal Society of London founded
1673	van Leeuwenhoek work taken to the Royal Society
1729	Achromatic lenses for spectacles were developed by Chester Moore Hall
1807	Achromatic lenses made commercially by Harmanus van Deyl
1849–1854	Early tissue staining methods with carmine, a naturally occurring dye by Goppart, Cohen and Hartig
1856	Introduction of the use of analine dyes
1858	Initial staining techniques developed for nerve cells by Gerlach
1880	First oil immersion objective lens introduced by Carl Zeiss Jena
1882	Acid fastness described by Ehrlich
1883	Introduction of the AFB staining technique by Ziehl and Neelsen
1884	Introduction of the Gram stain by Hans Christian Gram
1904	Principles of fluorescent microscopy seen by Kohler
1931	Ernst Ruska and Maximilian Knoll were credited with the invention of the electron microscope
1934	Phase contrast microscope developed by Zernike
1964	Resolution of 1nm was possible with electron microscopy
1994	M. Chalfie *et al*. succeed in expressing a naturally fluorescent protein, the now-famous green fluorescent protein (GFP), in living organisms, a major breakthrough for fluorescent microscopy
2007	Pro-Lab Diagnostics introduces the Poly Stainer™ in microbiology
2011	New regulations tighten the production and quality control of staining solutions

staining began. He treated the muscle with an extract obtained by macerating saffron in burnt wine. The saffron, taken from the styles and stigmas of the *Crocus sativus* flower, native to Spain, is still used to this day as a histology stain to show tissue structure. This method opened the door for many naturally occurring dyes to be experimented with for staining all manner of cells, structures and tissues.

In 1849, Goppert and Cohen used carmine with limited success. In 1854, Hartig continued the work with litmus, black ink and copper sulfate, and is credited as a front runner in the process of histological staining, later completed by Gerlach who actually made his most important discovery by accident; this led to a huge advance with the staining of nerve fibres and nerve cells using ammoniacal carmine. This spurred many researchers on, and today there are hundreds of differential staining techniques using numerous blends of pigments and dyes. These can be referenced in the International Colour Index, first published in 1925, containing over 27,000 individual products offering a valuable reference for the scientific, textile, paint, printing and plastic industries. Pigments and dyes can be acidic, basic, dispersing, direct, fluorescent, mordant and reactive, and can be used in many staining techniques such as direct, simple, indirect, progressive, regressive, vital, intra-vital, supra-vital, negative, impregnative, polychromatic and metachromatic.

A common example...

Perhaps one of the most widely used staining methods in microbiology is the Gram stain, and although one of the simplest staining methods used, it is one of the most fascinating polychromatic methods enabling initial differentiation based on colour and morphology.

Gram staining is named after the Danish microbiologist Hans Christian Gram, who initially researched the method in 1882 and published the first recommended method in 1884 (Table 1). It is still one of the most important staining techniques in microbiology, being one of the first tests performed for bacterial identification offering valuable diagnostic information.

Figure 4 shows the basic structure of Gram-negative and Gram-positive cell walls. The differences are exploited in Gram's method of staining. Gram-positive cells are able to retain a dye-mordant complex and so appear the same dark purple colour as the primary dye, usually crystal violet. Conversely, the dye-mordant complex can be leached out of Gram-negative cells by a solvent, or decolourizer/differentiator, and therefore these appear the same colour as the pink/red counterstain.

Care must always be taken; Gram staining is an art form, practised over many years by all microbiologists, and results can vary if care is not taken.

Around the same time that Gram introduced the Gram stain, the acid fastness of some bacteria was noted by Paul Ehrlich in 1882. A stain for Acid/Alcohol Fast Bacilli (AFBs or AAFBs), primarily for *Mycobacterium* species, was introduced by Ziehl and Neelsen in 1883; the ZN stain. This then became the classic method for staining *Mycobacterium tuberculosis*. This method requires heat to be applied to the

Figure 4. *Basic structure of Gram-negative and Gram-positive cell walls*

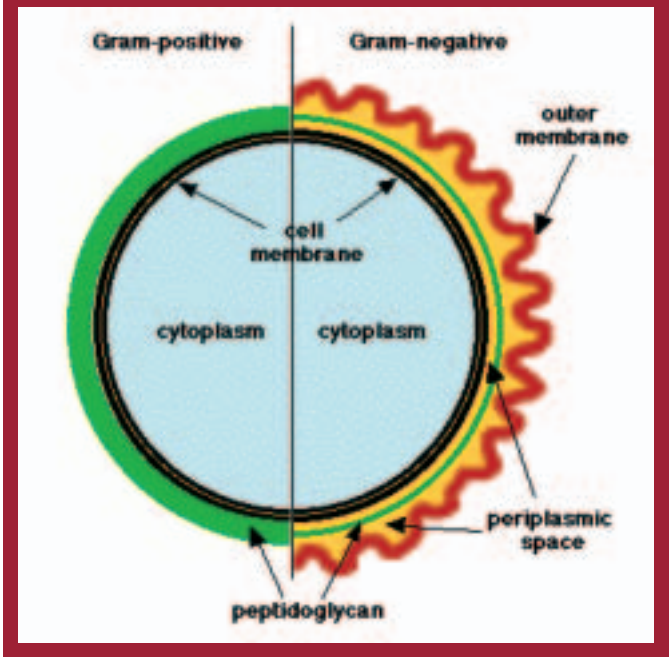


Figure 5. *Poly Stainer™ for automated staining procedures*

Figure 6. *Dyes and immersion oil used for microscopy of stained samples*

specimen slide, although a 'cold' alternative is available with the Kinyoun staining method.

Fluorescence microscopy was introduced early in the 20th century. Initially, observations were limited to specimens that fluoresce naturally, then fluorescent dyes for staining tissues and cells were investigated. During the 1940s, fluorescence microscopy became popular when Coons and Kaplan introduced a technique to label antibodies with a fluorescent dye to study antibody-antigen interactions. This led to major changes and important developments in the field of immune histochemistry. Then, in 1994, the discovery that really brought fluorescence microscopy to the forefront was when Chalfie *et al.* succeeded in expressing a naturally green fluorescent protein (GFP) in living organisms, leading to additional research for a whole new class of tagging methods.

The development of fluorescent microscopy also led to the auramine phenol stain becoming a popular choice for screening sample smears for AFBs, although all positives still require checking with the traditional ZN staining method. In 1931, Ernst Ruska and Maximilian Knoll were credited with the invention of the electron microscope, and by 1964 resolution of 1nm became possible.

Traditional hands-on or automation?

As workloads increase in busy laboratories we are presented with many challenges for more accurate results in a more timely fashion. This inevitably leads to the question of automation. Many staining methods can be, and have been, automated, offering accurate and consistent results, whilst freeing up valuable laboratory staff time. This automation can be achieved in all disciplines, and in microbiology the Poly Stainer™ offers automated staining for a variety of microbiological staining methods, such as Gram staining, TB staining, *Cryptosporidium* staining and *Trichomonas* staining.

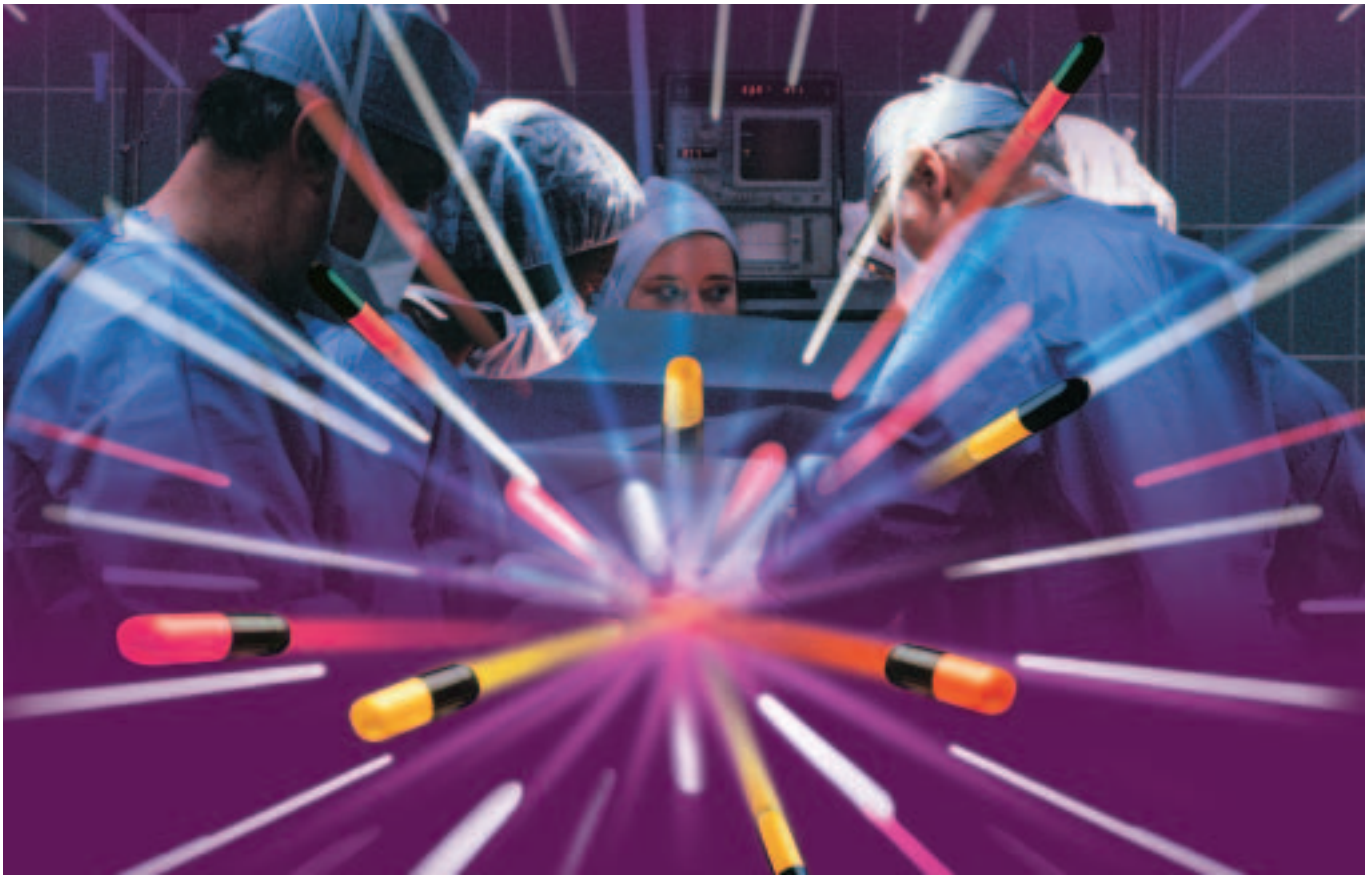
Watch your oil!

With the development of the first oil immersion lens in 1880 by Carl Zeiss Jena, the use of immersion oil was also required. Magnification was improved with the use of oil immersion lenses and the image quality could be directly related to the optical properties of the immersion oil used. The use of oil with virtually the same refractive index as the glass used in the microscopy slides (1.513) increases the image resolution. In the absence of suitably matched oil, some of the light rays from the sample do not reach the objective due to refraction at the glass-air interface.

Conclusion

A time span of almost 360 years has seen major advances in microscopy from its humble beginnings with the van Leeuwenhoek microscope, to the scanning electron microscope. Throughout this time, many staining techniques have been developed and this tradition remains strong with some of the most tried and trusted methods, as described above, still used today.

Mark Reed
Pro-Lab Diagnostics



Antimicrobial resistance — a real and present danger

During 2013 the UK's political spotlight was fixed on the issue of antimicrobial resistance (AMR). In March, the UK Chief Medical Officer published a report on the threat of AMR and infectious disease; the Science Minister highlighted the issue in his speech to the G8 Science Ministers in June; and towards the end of the year the House of Commons Science and Technology Select Committee requested written and oral evidence in an inquiry on this subject, to which S*f*AM has contributed. Executive Committee Member John Threlfall appeared before the Select Committee in December.

This issue will run for some time and the Society will be called upon to bring Members' expertise to bear on the debate. If this topic is of professional interest to you and you are a Member of S*f*AM, would you be willing to be involved in future activities? Please get in touch with the team at communications@sfam.org.uk.

How has AMR developed during the past decade?

There has been widespread emergence of resistance to third-generation cephalosporins in both pathogenic and non-pathogenic bacteria; in particular, in the UK, there has been increasing incidence of community outbreaks of *E. coli* with resistance to third-generation cephalosporins, resulting in numerous fatalities. In parallel, has been the increasing occurrence in poultry of commensal *E. coli* with cephalosporin resistance, posing a dual threat of emerging cephalosporin-resistant reservoirs of *E. coli* with the capability of spreading their resistance genes to pathogenic bacteria in the animal and human gut environments.

On the Indian subcontinent, resistance to **carbapenem** antibiotics in already multiple drug-resistant bacteria has emerged, resulting in almost untreatable infections. And recently, carbapenemase-producing *Acinetobacter* spp., *Salmonella enterica* and *E. coli* have been reported in European food animals.

Meticillin-resistant *Staphylococcus aureus* (MRSA) strains with varying levels of **vancomycin resistance**, from intermediate to resistant, have been detected. Of particular concern, the past decade has seen essentially untreatable cases of tuberculosis and gonorrhoea where the pathogen is extensively resistant to drugs.

What are some of the gaps in our knowledge about AMR?

Knowledge of the **distribution and exchange of genes** in the wider environment and what drives these processes is severely lacking. This includes the extent of the resistance reservoir in human and animal commensal microbiota of the gut, skin, oral cavity, etc. in healthy individuals, even prior to any antibiotic exposure.

The impact of antibiotic exposure on

commensal microbiota in the short and long term is unknown, as is the distribution of genes conferring resistance to critically important antibiotics, or antibiotics of last resort.

In addition, the exchange and transmission of resistance genes in both pathogenic and non-pathogenic organisms from humans to animals, and *vice versa*, particularly involving animals that are reared for food, has not been quantified.

Increasing **globalization** of our lives compounds existing problems. International travel and food imports have contributed to the worldwide spread of resistant strains, e.g., NDM-1, resulting in untreatable infections in economically developed countries in Europe and North America. The extent of spread via these routes is not fully understood.

There remains a question about the impact of **reducing the selective pressure** on organisms, in both the human and veterinary sphere. For example, little is known about the effect of the 'off-label' use of antibiotics in veterinary medicine — could reducing such usage, as well as other cases of overprescribing, particularly in human medicine, reduce the burden of resistance?

The use of **metals**, particularly silver, in wound dressings and other antiseptic applications brings a new set of uncertainties. Resistance to silver in clinical isolates is seldom seen, but exposure around silver mines and in other non-clinical settings is documented to confer resistance. A number of metal resistances are co-transmitted with AMR on plasmids/transposons, so the use of silver as an antimicrobial treatment in many different environments has the potential to select for resistant strains and could indirectly drive AMR — this requires further investigation.

Is there sufficient research and investment into new antibiotics or other treatments and methods to ensure continued protection against infection? If not, how could this be rectified?

In the past, pharmaceutical companies have invested heavily in the development of formulations that are both effective antibiotics and also discourage/minimize the evolution of resistance. That is no longer the case and there is relatively little R&D going on in this area. A primary reason appears to be the greater financial rewards in developing other classes of drugs. This puts the onus on the **UK Government to fund antimicrobial drug development outside of the pharmaceutical companies and/or incentivize this work within the industry**.

A recent *Journal of Antimicrobial Chemotherapy* article has provided detailed analysis of the proportion of total infection research into AMR (bit.ly/JAC_Head). The paper also outlines some recommendations for the future direction of R&D. In summary: despite the rapid emergence of AMR, **the proportion of UK infection-research spend targeting this critical area remains small (3.9%; £102 million of £2.6 billion total spend**). Mean annual funding ranged from £1.9 million in 1997 to £22.1 million in 2009. The study concluded that whilst this is an encouraging indication of increased emphasis and investment in this important area of research, the UK Government must continue to fund AMR research in a sustained and targeted manner.

Understanding the drivers selecting for resistance, which may include as yet unknown factors, is also vitally important. **A quantitative risk assessment of the effects of different control measures in human and animal populations** would provide the basis from which to develop new drugs that have the potential for greater longevity.

It is also worth noting that research under the banner of '**One Health, One Medicine**', i.e., dual research in human and veterinary contexts, has the potential to take this complex research into its proper social setting.

What measures (including behavioural change) have been most effective in controlling the spread of resistant pathogens, and could such measures be used to control other pathogens?

The continued promotion of responsible use of medicines in agriculture is undertaken by the Responsible Use of Medicines in Agriculture Alliance (RUMA) in the UK, the European Platform for the Responsible use of Medicines in Agriculture (EPRUMA) and the OIE (World Organization for Animal Health). Such activities have included the withdrawal of antibiotics as growth promoters in the EU since 2006, which has been seen as highly effective in controlling the appearance and spread of some, mainly Gram-positive, organisms in food animals and hence their spread to humans through the food chain. Similar controls for the prophylactic use of certain antibiotics in food production may have some effect, but such measures need to be balanced against possible effects on animal health and on food production. A typical example is in weaner pigs where antibiotic treatment is regarded as a crucial preventative measure. Parallel controls on the use of prophylactic antibiotics in humans should also be considered.

EU-wide legislative controls to reduce the occurrence of pathogenic bacteria such as *Salmonella* Enteritidis and *Salm*. Typhimurium in poultry have been highly effective in reducing their occurrence, including AMR strains of these serovars. Such bacteria are frequently resistant to commonly used antibiotics as a consequence of antibiotic use in certain food production animals.

The prevention of infectious disease through appropriate **hygiene and biosecurity**, through the use of **vaccines** where available, and by **appropriate nutrition and housing of animals**, reduces the need for antibiotic use on farms.

High hygiene standards during slaughter, processing and preparation of animal products (including dairy and eggs) is vital. This means **education** of food industry workers on compliance with legislation, and enforcement where breaches have occurred, is effective to reduce the spread of foodborne pathogens. Food hygiene **campaigns** aimed at the general population may also be effective in reducing the transmission of AMR bacteria through the food chain.

Many medical doctors prescribe antibiotics unnecessarily, often under pressure from patients; and, under pressure from farmers, veterinarians may prescribe antibiotics as a substitute for poor husbandry, thereby perpetuating the occurrence of AMR in the food chain. **Education** of prescribing doctors and veterinarians is required.

Many of the problematic AMR strains in cases of human infection are isolated

from chronic wounds. **Careful management of patients** is therefore particularly important to ensure that they do not become a source of infection. Frequent debridement of the wound using non-invasive techniques (taking care because aerosols can be produced), then topical application of antiseptic dressings until bacterial numbers are reduced is effective.

What global co-ordination and action is required to fight antimicrobial resistance and is the UK contributing enough towards cross-border initiatives?

There have been many initiatives to combat AMR at UK, EU and global levels, particularly in respect of resistance in food animals. Some key initiatives, both past and present, are listed in the box to the right.

The European Medicines Agency (EMA), the European Centre for Disease Prevention and Control (ECDC), and the European Food Safety Authority (EFSA) have all realized the importance afforded to the control of infections caused by AMR bacteria, and many collaborative actions are underway. More proactive collaborations between appropriate UK agencies and these bodies would be both welcome and desirable.

Antibiotics are used freely in Far East countries in both human and veterinary medicine, often with formulations that have not been subject to proper regulatory controls. This usage has undoubtedly contributed to the appearance of, and subsequent worldwide spread of, organisms resistant to critically important antimicrobials (CIAs) and last-resort antibiotics, and must be brought under global regulatory control.

When 'new' AMR strains emerge in humans or animals, appropriate control measures should be proactively implemented at national and international levels to prevent such strains becoming widespread.

Environment pollution around factories producing antibiotics has been reported; global regulation and compliance with respect to groundwater and drinking water contamination is essential.

Key initiatives

UK

1970: The Swann recommendations — banning the use of antibiotic feed additives in animal husbandry in the UK

2009: Chief Medical Officer Report — recommendation that there should be a ban on the use of certain types of antibiotics (quinolones and cephalosporins) in animals, in order to protect their activity in humans

2013: Chief Medical Officer Report — recommendation for national approach to tackling antimicrobial resistance to be managed jointly between DH and Defra

2013: House of Commons — call to phase out the prophylactic use of antibiotics in intensive livestock farming in the UK (similar to that made in the European Parliament in 2011)

European Union

2004–2013: EMA — guidance on pre-approval information for registration of new veterinary medicinal products for food-producing animals with respect to antimicrobial resistance

2006: Ban of use of growth promoters in animal feeds — regulation 1831/2003/EC on additives for use in animal nutrition

2011: European Food Safety Authority (EFSA) — scientific opinion on the public health risks of bacterial strains producing ESBLs and/or AmpC β-lactamases in food and food-producing animals; recommendations for controls on the use of cephalosporins in food animals

2011: European Parliament — call to phase out the prophylactic use of antibiotics in livestock farming in the EU

2011: SANCO strategy on Antimicrobial Resistance — five-year strategy presented on European Antibiotic Awareness Day, 18 November 2011

2011: WHO Europe — strategy on AMR for tackling AMR from a food safety perspective

2013: European Food Safety Authority (EFSA) — scientific opinion on carbapenem resistance in the food chain; recommendations to prevent spread of carbapenemase-producing bacteria in food animals

Global

WHO/FAO/OIE achievements on AMR since 1997

- International collaboration established.
- 15 plus expert meetings and consultations.
- Codex and OIE: normative work.
- FAO and OIE: practical guidance and capacity building.
- WHO: raise public awareness, monitoring, leading the debate.

2007, 2009, 2011: WHO — list of critically important antimicrobials (CIAs) e.g., quinolones/fluoroquinolones, 3rd/4th generation cephalosporins, carbapenams, aminoglycosides

2007–2010: Codex ad hoc Intergovernmental Task Force on AMR — outcome: Guidelines for the Risk Analysis of Foodborne Antimicrobial Resistance

2008–2013: Codex Alimentarius Strategic Plan — develop guidance for safe and prudent non-human antimicrobial usage for containment of resistance

2010: OIE — list of antimicrobials of veterinary importance published

2011: European Parliament — calls for the European Commission to make legislative proposals to phase out the prophylactic use of antibiotics in livestock farming in the EU (European Parliament, 27 October 2011)


Nancy Mendoza (left) and **John Threlfall** (right)

bio**Focus**

Mark Downs talks about the problems associated with the production of algal biofuels

www.societyofbiology.org

In the December 2013 issue of *Microbiologist* (Vol. 14 No. 4) I wrote about future predictions for the Society of Biology. My gazing into this crystal ball coincided with predictions at the Society about the future of energy production, specifically algal biofuels.

The title of our discussion event, '*Full Bloom or Dead in the Water*', was testament to the varied opinions about whether algae has any potential as an energy source. The sustainability of land-based biofuels, especially those based on food plants, has been widely questioned, so energy from algae is an appealing prospect. The plot thickens, however, when we consider the practicalities — we still have large hurdles to overcome if we're going to scale-up production.

Part of the appeal of algae is that it could theoretically be used to produce liquid fuels. Transport accounts for a quarter of UK carbon emissions, but is proving the hardest part of the economy to decarbonize. In all European countries, 10% of transport energy must come from renewable sources by 2020, so the stakes are high.

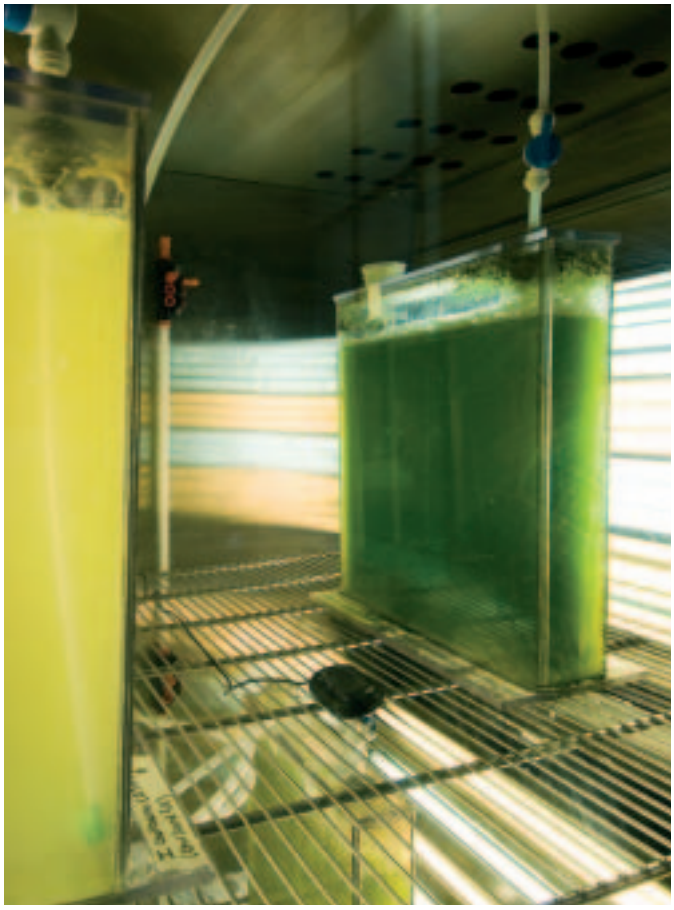
A major limitation to producing biofuels is the use of land. For 50% of the USA's fuel requirements with corn oil, you'd need 846% of its available crop area. With a growing population and changing diets, land can't simply be diverted away from food production.

Algae must be grown in the hundreds of thousands to millions of tonnes per year scale globally to replace current petroleum usage. Yet the current estimate of global production of dry algal biomass is under 10,000 tonnes per year, including algae grown for other purposes.

The event's Chair, Michele Stanley, director of NERC's Algal Bioenergy Special Interest Group, shared a prediction that 447 TJ of energy can be produced by macroalgae by 2020. That's about 0.2% of current road fuel demands.

Scale isn't the only challenge we need to overcome; even with recent increases in the price of oil, algal biofuels are currently unable to compete economically. It was reassuring to hear Oliver Chadwick from the Department for Transport say that an important aspect of the economics is 'cost per tonne of carbon saved'.

One approach to the problems of scale and cost is to combine production of algal biofuels with another process. It



could even be used to solve waste problems. Fish farms, for example, are serious polluters, with waste nitrogen damaging ecosystems. Algae could be used to clean up the waste, and then be converted into biofuel.

Another option is to use algae to produce higher value products, such as pharmaceuticals, and produce bioenergy as a 'by-product'.

Speaker Andrew Spicer, from the algal bioenergy company Algenuity, looked beyond the technological challenges in his predictions of the future. Microalgae are starting to enter the genome-editing realm. Metabolic engineering of biochemical pathways could achieve improved productivities, lower costs and improved energy balance. If we are accurately altering individual nucleotide bases, will this still be classed as genetic modification? It is under current legislation. Will it be acceptable to grow these algae in large-scale, outdoor facilities?

The technology may not be here yet, but part of paving the way is to open the ethical debate.

The event was part of our Policy Lates discussion series, evening events open to everyone tackling science policy issues. Having dedicated the latest event to predicting the future of algal biofuels we are planning a future discussion on '*Should Scientists Predict the Future*?'

Dr Mark Downs, PhD, FSB
Chief Executive, Society of Biology

journalWatch

News about the Society's journals

Highlighted Articles from the SfAM journals

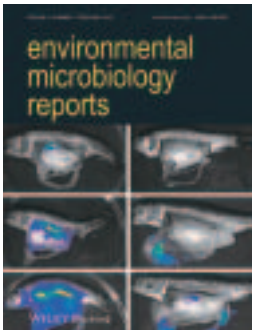
Environmental Microbiology

An improved cell separation technique for marine subsurface sediments: applications for high-throughput analysis using flow cytometry and cell sorting.
Morono *et al*.

Development of an improved technique for separating microbial cells from marine sediments, and standardization of a high-throughput and discriminative cell enumeration method were conducted. We separated microbial cells from various types of marine sediment and then recovered the cells using multilayer density gradients of sodium polytungstate and/or Nycodenz, resulting in a notably higher per cent recovery of cells than previous methods. We also demonstrated that sedimentary microbial cells can be efficiently collected using a cell sorter. The combined use of our new cell separation and FCM/cell sorting techniques facilitates high-throughput and precise enumeration of microbial cells in sediments, and is amenable to various types of single-cell analyses, thereby enhancing our understanding of microbial life in the largely uncharacterized deep subseafloor biosphere.
bit.ly/EMI_Morono

Marine biofilms on artificial surfaces: structure and dynamics.
Salta *et al*.

The search for new antifouling (AF) coatings that are environmentally benign has led to renewed interest in the ways that microorganisms colonize substrates in the marine environment. This review covers recently published research on the global species composition and dynamics of marine biofilms, consisting mainly of bacteria and diatoms found on man-made surfaces including AF coatings. Marine biofilms directly interact with larger organisms (macrofoulers) during colonization processes; hence, recent literature on understanding the basis of the biofilm/macrofouling interactions is essential and is also reviewed here.
bit.ly/EMI_Salta



Environmental Microbiology Reports

Strict vegetarian diet improves the risk factors associated with metabolic diseases by modulating gut microbiota and reducing intestinal inflammation.
Kim *et al*.

Low-grade inflammation of the intestine results in metabolic dysfunction, in which dysbiosis of the gut microbiota is intimately involved. Dietary fibre induces prebiotic effects that may restore imbalances in the gut microbiota; however, no clinical trials have been reported in patients with metabolic diseases. Here, six obese subjects with type 2 diabetes and/or hypertension were assigned to a strict vegetarian diet (SVD) for 1 month, and blood biomarkers of glucose and lipid metabolisms, faecal microbiota using 454-pyrosequencing of 16S ribosomal RNA genes, faecal lipocalin-2 and short-chain fatty acids were monitored. An SVD reduced body weight and the concentrations of triglycerides, total cholesterol, low-density lipoprotein cholesterol and haemoglobin A1c, and improved fasting glucose and postprandial glucose levels. An SVD reduced the *Firmicutes*-to-*Bacteroidetes* ratio in the gut microbiota, but did not alter enterotypes. An SVD led to a decrease in the pathobionts such as the *Enterobacteriaceae* and an increase in commensal microbes such as *Bacteroides fragilis* and *Clostridium* species belonging to clusters XIVa and IV, resulting in reduced intestinal lipocalin-2 and short-chain fatty acid levels. This study underscores the benefits of dietary fibre for improving the risk factors of metabolic diseases and shows that increased fibre intake reduces gut inflammation by changing the gut microbiota.
bit.ly/EMR_Kim

Assembly-free metagenomic analysis reveals new metabolic capabilities in surface ocean bacterioplankton.
Luo and Moran.

Uncovering the metabolic capabilities of microbes is key to understanding global energy flux and nutrient transformations. Since the vast majority of environmental microorganisms are uncultured, metagenomics has become an important tool to genotype the microbial community. This study uses a recently developed computational method to confidently assign metagenomic reads to microbial clades without the requirement of metagenome assembly, by comparing the evolutionary pattern of nucleotide sequences at non-synonymous sites between metagenomic and orthologous reference genes. We found evidence for new, ecologically relevant metabolic pathways in several lineages of surface ocean bacterioplankton using the Global Ocean Survey (GOS) metagenomic data, including assimilatory sulfate reduction and alkaline phosphatase capabilities in the alphaproteobacterial SAR11 clade, and proteorhodopsin-like genes in the cyanobacterial genus *Prochlorococcus*. These findings raise new hypotheses about microbial roles in energy flux and organic matter transformation in the ocean.
bit.ly/EMR_Luo-Moran

Journal of Applied Microbiology

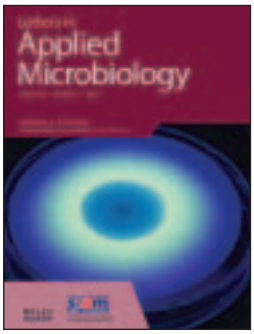
Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related microorganisms.
Kerekes *et al*.

Biofilm formation on foods and food industrial equipment is a serious problem causing food spoilage and emergence of foodborne diseases. The aim of this study was to investigate the effect of clary sage,

juniper, lemon and marjoram essential oils (EOs), and their major components on the formation of bacterial and yeast biofilms, and on the inhibition of AHL-mediated quorum sensing (QS). The EOs and components used seem to be good candidates for the prevention of biofilm formation and inhibition of the AHL-mediated QS mechanism. This article highlights the importance of studying EOs as potential disinfectants and food preservatives.
bit.ly/JAM_Kerekes

Fatty acid oxidation products ('green odour') released from perennial ryegrass following biotic and abiotic stress, potentially have antimicrobial properties against the rumen microbiota resulting in decreased biohydrogenation.
Huws *et al.*

In this experiment, we investigated the effect of 'green odour' products typical of those released from fresh forage postabiotic and biotic stresses on the rumen microbiota and lipid metabolism. Hydroperoxyoctadecatrienoic acid, T and HPT released due to plant stress potentially have an antimicrobial effect on the rumen microbiota, which may explain the decreased biohydrogenation *in vitro*. These data suggest that these volatile chemicals may be responsible for the higher summer *n-3* content of bovine milk.
bit.ly/JAM_Huws
See also: bit.ly/SfAM_CutGrassOmega3



Letters in Applied Microbiology

Cytotoxicity and mycotoxin production of shellfish-derived *Penicillium* spp., a risk for shellfish consumers.
Geiger *et al*.

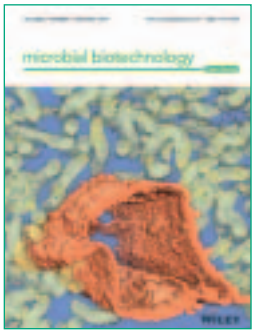
In order to assess the putative toxigenic risk associated with the presence of fungal strains in shellfish-farming areas, *Penicillium* strains were isolated from bivalve molluscs and from the surrounding environment, and the influence of the sample origin on the cytotoxicity of the extracts was evaluated. Extracts obtained from shellfish-derived penicillia exhibited higher cytotoxicity than the others.

Penicillium strains isolated from bivalve molluscs produce extracts exhibiting a higher cytotoxicity than extracts from *Penicillium* strains isolated from the surrounding marine environment. The use of a mussel-based medium for cultures of some shellfish-derived strains enhances the cytotoxicity of extracts when compared with classical media. The production of cytotoxic compounds and of the mycotoxin patulin on such a host-derived medium highlights a potential health risk for shellfish consumers.

bit.ly/LAM_Geiger
See also: bit.ly/SfAM_ShellfishMycotoxin

Production of melanin pigment from *Pseudomonas stutzeri* isolated from red seaweed *Hypnea musciformis*.
Kumar *et al*.

This investigation reports a marine *Pseudomonas stutzeri* strain HMGM-7 [MTCC 11712] that produces significant quantities of melanin (6.7 g/l) in a sea-water medium without the supplementation of L-tyrosine. Confirmation of the produced melanin was carried out by various chemical and physical characterization studies. The isolated melanin may find potential application for use in cosmetic and/or pharmaceutical industries.
bit.ly/LAM_Kumar



Microbial Biotechnology

Recombinant DNA production of spider silk proteins.
Tokareva *et al*.

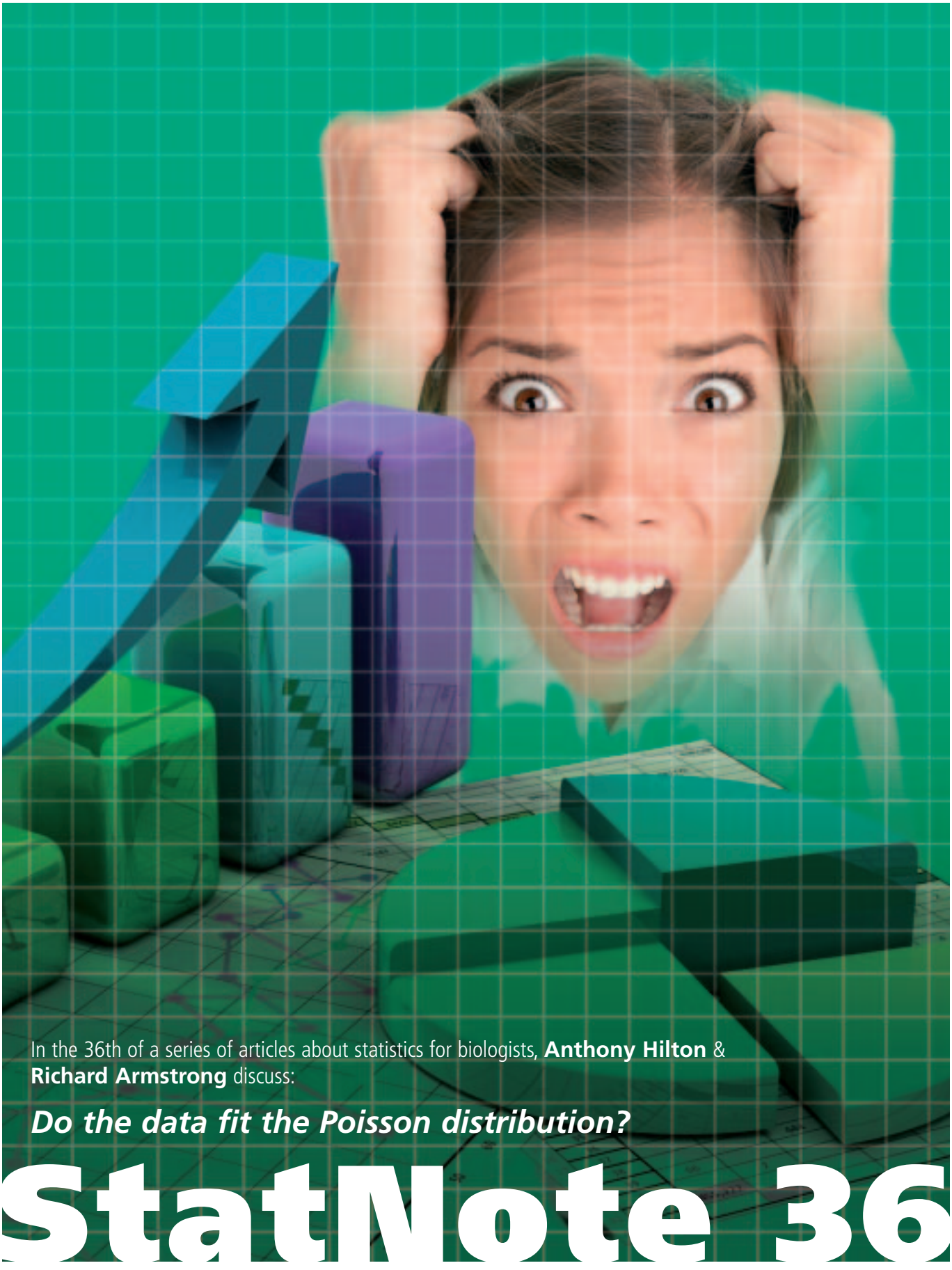
Spider dragline silk is considered to be the toughest biopolymer on Earth due to an extraordinary combination of strength and elasticity. Moreover, silks are biocompatible and biodegradable protein-based materials. Recent advances in genetic engineering make it possible to produce recombinant silks in heterologous hosts, opening up opportunities for large-scale production of recombinant silks for various biomedical and material science applications. We review the current strategies to produce recombinant spider silks.
bit.ly/MBT_Tokareva

Bioremediation: a genuine technology to remediate radionuclides from the environment.
Prakash *et al*.

Radionuclides in the environment are a major human and environmental health concern. Bioremediation has been viewed as the ecologically responsible alternative to environmentally destructive physical remediation. Microorganisms carry endogenous genetic, biochemical and physiological properties that make them ideal agents for pollutant remediation in soil and groundwater. Attempts have been made to develop native or genetically engineered (GE) microbes for the remediation of environmental contaminants including radionuclides.

Microorganism-mediated bioremediation can affect the solubility, bioavailability and mobility of radionuclides. Therefore, we aim to unveil the microbial-mediated mechanisms for biotransformation of radionuclides under various environmental conditions as developing strategies for the waste management of radionuclides. A discussion follows of '-omics'-integrated genomics and proteomics technologies, which can be used to trace the genes and proteins of interest in a given microorganism towards a cell-free bioremediation strategy.
bit.ly/MBT_Prakash

Melissa McCulloch
Wiley-Blackwell



In the 36th of a series of articles about statistics for biologists, **Anthony Hilton** & **Richard Armstrong** discuss:

Do the data fit the Poisson distribution?

StatNote 36

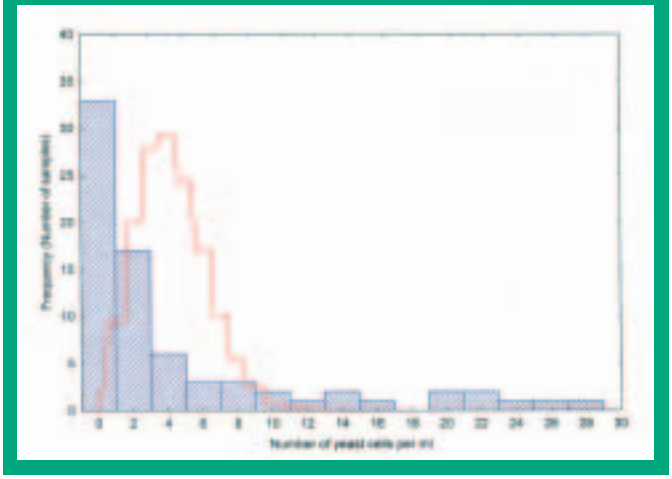
Table 1. Observed and expected frequencies for the counts of yeast cells. Goodness-of-fit tests of fit to the Poisson distribution: χ^2 (all categories) = 109.98 (P < 0.01); KS test = 0.51 (P < 0.01); Variance (V) = 48.67, Mean (M) = 4.19, V/M = 11.62

Category (upper limits) of yeast cell counts	O	E	O – E
< = 0	33	1.13976	31.8602
2.0	17	14.76081	2.2392
4.0	6	28.53095	-22.5309
6.0	3	20.74228	-17.7423
8.0	3	7.76709	-4.7671
10.0	2	7.76095	0.2390
12.0	1	0.26680	0.7332
14.0	2	0.02887	1.9711
16.0	1	0.00234	0.9977
18.0	0	0.00015	-0.0001
20.0	2	0.00001	2.0
22.0	2	0	2.0
24.0	1	0	1.0
26.0	1	0	1.0
< Infinity	1	0	1.0

O = Observed frequency of yeast cells in a sample, E = Expected frequency of yeast cells based on Poisson distribution

Figure 1. *Histogram illustrating the observed distribution of counts of yeast cells (blue histogram) and the predicted Poisson distribution (red line).*

The histogram suggests that the Poisson distribution significantly underestimated the frequency of samples which had zero yeast cells present, significantly overestimated the frequency of samples with between 2 and 8 yeast cells per sample and underestimated the number of samples with greater than 10 yeast cells per sample



Introduction

In previous StatNotes, many of the statistical tests described rely on the assumption that the data are a random sample from a normal or Gaussian distribution. These include most of the tests in common usage such as the 't' test (StatNote 3, Hilton & Armstrong, 2005b), the various types of analysis of variance (ANOVA) (e.g., StatNote 9, Hilton & Armstrong, 2007a; StatNote 11, Hilton & Armstrong, 2007b), and Pearson's correlation coefficient ('r') (StatNote 14, Hilton & Armstrong, 2008).

In microbiology research, however, not all variables can be assumed to follow a normal distribution. Yeast populations, for example, are a notable feature of freshwater habitats, and representatives of over 100 genera have been recorded (Nagahama *et al.*, 2006). Most common are the 'red yeasts' such as *Rhodotorula*, *Rhodosporidium* and *Sporobolomyces*, and 'black yeasts' such as *Aurobasidium pelculans*, together with species of *Candida*. Despite the abundance of genera and species, the overall density of an individual species in freshwater is likely to be low and hence, samples taken from such a population will contain very low numbers of cells. A rare organism living in an aquatic environment may be distributed more or less at random in a volume of water and therefore, samples taken from such an environment may result in counts which are more likely to be distributed according to the Poisson than the normal distribution (El Shaarawi *et al.*, 1981). The Poisson distribution was named after the French mathematician Siméon Poisson (1781−1840) and has many applications in biology, especially in describing rare or randomly distributed events, e.g., the number of mutations in a given sequence of DNA after exposure to a fixed amount of radiation or the number of cells infected by a virus given a fixed level of exposure. This StatNote describes how to fit the Poisson distribution to counts of yeast cells in samples taken from a freshwater lake.

Scenario

Background

Studies of the density of aquatic yeasts or bacteria in large bodies of water usually involve the collection of a number of water samples taken at various locations over a period of time. To use these data to estimate the overall density of a specific organism, however, assumptions are often made concerning how the samples may be representative of the body of water as a whole and especially whether density is likely to follow a particular statistical distribution. One of the first such studies (Phelps, 1908) assumed that coliform bacteria were distributed randomly in small volumes of water. Later, Greenwood and Yule (1917) suggested that numbers of bacteria in such samples could be distributed according to the Poisson distribution. Subsequently, Fisher *et al.*, (1922) concluded that the Poisson distribution was a good fit to bacterial counts obtained under experimental conditions and moreover, if a Poisson distribution did not fit the data adequately, then a different, *viz.*, the negative binomial distribution, could be used as an alternative. Hence, the total variation in numbers of an organism in small-volume water samples is often considered to be made up of two components: (1) a random component described by the Poisson distribution and (2) a non-random component representing deviations from a Poisson distribution (El Shaarawi *et al.*, 1981).

Methodology

To fit the Poisson distribution, the number of yeast cells per sample was estimated in 75 x 5ml samples of water collected from a freshwater lake. 1ml subsamples were then taken from each of the larger samples, the original suspension being mixed thoroughly before taking the subsample. The number of yeast cells per sample will be low so dilution of the sample before counting is usually not required. The number of yeast cells was counted using an improved Neubauer

haemocytometer which enables the total number of cells per ml to be estimated. Hence, the data comprise the number of yeast cells present in each of the 75, 5ml samples and the frequency distribution of these counts is listed in Table 1 and illustrated in Figure 1.

How is the analysis carried out?

A Poisson distribution was fitted to the numbers of yeast cells using STATISTICA software (Statsoft Inc., 2300 East 14th St, Tulsa, OK, 74104, USA). To fit the Poisson distribution, a method similar to that described in StatNote 1 to fit the normal distribution was used (Hilton & Armstrong, 2005a). First, the variable, i.e., number of yeast cells, is divided into frequency classes, 15 classes being used for the present data. Second, the Poisson distribution is used to calculate the expected numbers of yeast cells per sample. Hence, if cells are distributed at random in the body of water as a whole, the probability (P) of finding samples with 0, 1, 2, …, n cells is given by the Poisson distribution. Third, the observed and expected values are compared using either a chi-square (χ^2) 'goodness-of-fit' or a Kolmogorov–Smirnov (KS) test (StatNote 35, Hilton & Armstrong, 2013). In a population having a Poisson distribution, the population mean is equal to the population variance and therefore, a randomly distributed population would have a variance/mean (V/M) ratio of 1. If a cell is distributed more uniformly, however, there is a greater than random probability of finding only a few individual cells in most samples and such a distribution would have a lower variance than a Poisson. Hence, a V/M ratio less than 1 would indicate a uniform or regular distribution of cells. By contrast, if a cell is clustered, there is a greater than random probability of finding many cells in a few samples and such a distribution would have a greater variance than a Poisson. Hence, a V/M ratio greater than 1 indicates clustering of cells. The significance of departure of the V/M ratio from unity can be tested by a 't' test or by a chi-square (χ^2) test (Armstrong, 1993).

Interpretation

In the present example, KS = 0.51 ($P < 0.01$) and $\chi^2 = 109.98$ ($P < 0.001$) both of which suggest a highly significant deviation from the Poisson distribution. In addition, Figure 1 suggests that the Poisson distribution significantly underestimated the frequency of samples which had zero yeast cells present, significantly overestimated the frequency of samples with between 2 and 8 yeast cells, and underestimated the number of samples with greater than 10 yeast cells. These data suggest that the yeasts were not distributed at random within this body of water but rather exhibited clustering, i.e., the non-random component predominated in this population. In addition, in the present example, a V/M ratio of 11.62 was obtained confirming the departure from randomness towards aggregation or clustering. A disadvantage of using the Poisson distribution to assess spatial or temporal patterns of an organism is that the results are markedly affected by sample size, i.e., the volume of a sample (Armstrong, 1993). To overcome this problem, samples of increasing volume may be taken and the effect of volume size on the distribution of an aquatic organism systematically investigated (Armstrong, 1993). Alternative methods of studying the spatial pattern of an organism will be described in future StatNotes.

Conclusion

If the data comprise measurements of the density of a rare microbe, such as a yeast or bacterium in an aquatic environment, the data are unlikely to follow a normal distribution. To estimate density in such circumstances, the Poisson is one possible type of distribution that can be used to fit the data. In a Poisson distribution, the V/M ratio is unity which can be used as an index of spatial or temporal pattern. In the present example, the analysis suggests that the Poisson distribution did not fit the data and hence, that the yeasts investigated were not randomly distributed in the body of water. Moreover, the V/M ratio suggests that the yeast cells were clustered suggesting an alternative distribution, *viz.*, a negative binomial, may provide a better fit to the data. Fitting the negative binomial distribution will be described in the next StatNote.

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Anthony Hilton[1] and Richard Armstrong[2]

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2013 *Environmental Microbiology* lecture report

Applying bacterial metabolism using engineering principles

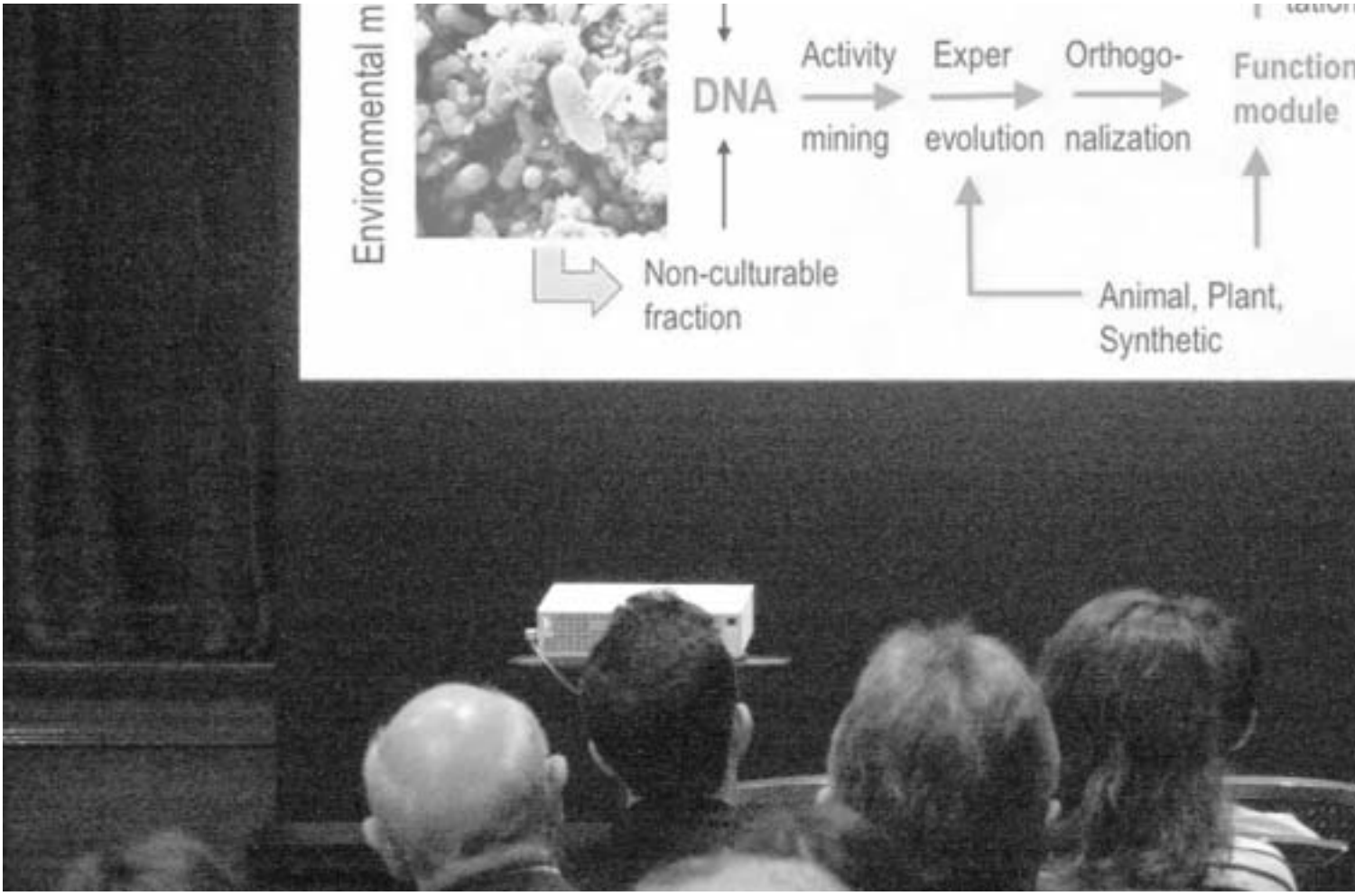
Held at the Institute of Civil Engineers, 1 George Street, London, on 28 October 2013

The 2013 SfAM *Environmental Microbiology* lecturer was Professor Victor de Lorenzo of the National Centre of Biotechnology, Madrid. He has chosen to think like an engineer in order to harness biological systems for the biomonitoring, bioremediation and valorization of chemical pollution in the environment.

Attendees at the lecture (which is available to watch online at bit.ly/EMI_Lecture_2013) were offered an insight into this ingenious approach, where bacteria and genetic elements are treated as modular building blocks that have the potential to be taken off the shelf and combined with a particular task in mind. It has been possible, Victor de Lorenzo says, to create "*something that nature has not invented before*", to improve on nature, even.

The approach shows that it is not always necessary to start from first principles to alter what is achievable in the

Professor Victor de Lorenzo receiving the lecture award from Vicky Johnson, Associate Editorial Director for Life Sciences at Wiley-Blackwell



application of bacterial metabolism. By combining the activities of individual organisms in a three-dimensional consortium, it has been possible to cherry-pick modules that together facilitate the degradation of environmental pollutants. In this way, scientists, thinking like engineers, are no longer at the behest of evolution, nor are they required to undertake lengthy activities to unpick the details of each biological tool in order to work from first principles.

Bacteria have adapted to grow on new carbon compound substrates — we see this in soil bacteria found in the vicinity of chemical industry contaminated sites. This immediately suggests the possibility of using this ability to our advantage in order to, for example, effect clean-up operations to reclaim brownfield sites for development.

Starting with *Pseudomonas putida* KT2440 — a strain isolated in Japan in the 1970s and selected for its ability to grow on a carbon compound called meta-toluate — Victor de Lorenzo described the process of stripping down and building up the bacterium using specially designed genetic building bricks to introduce new characteristics. N.B., Ps. *putida* is a good example since it is generally regarded as safe, grows on a wide variety of organic carbon sources and resists solvents.

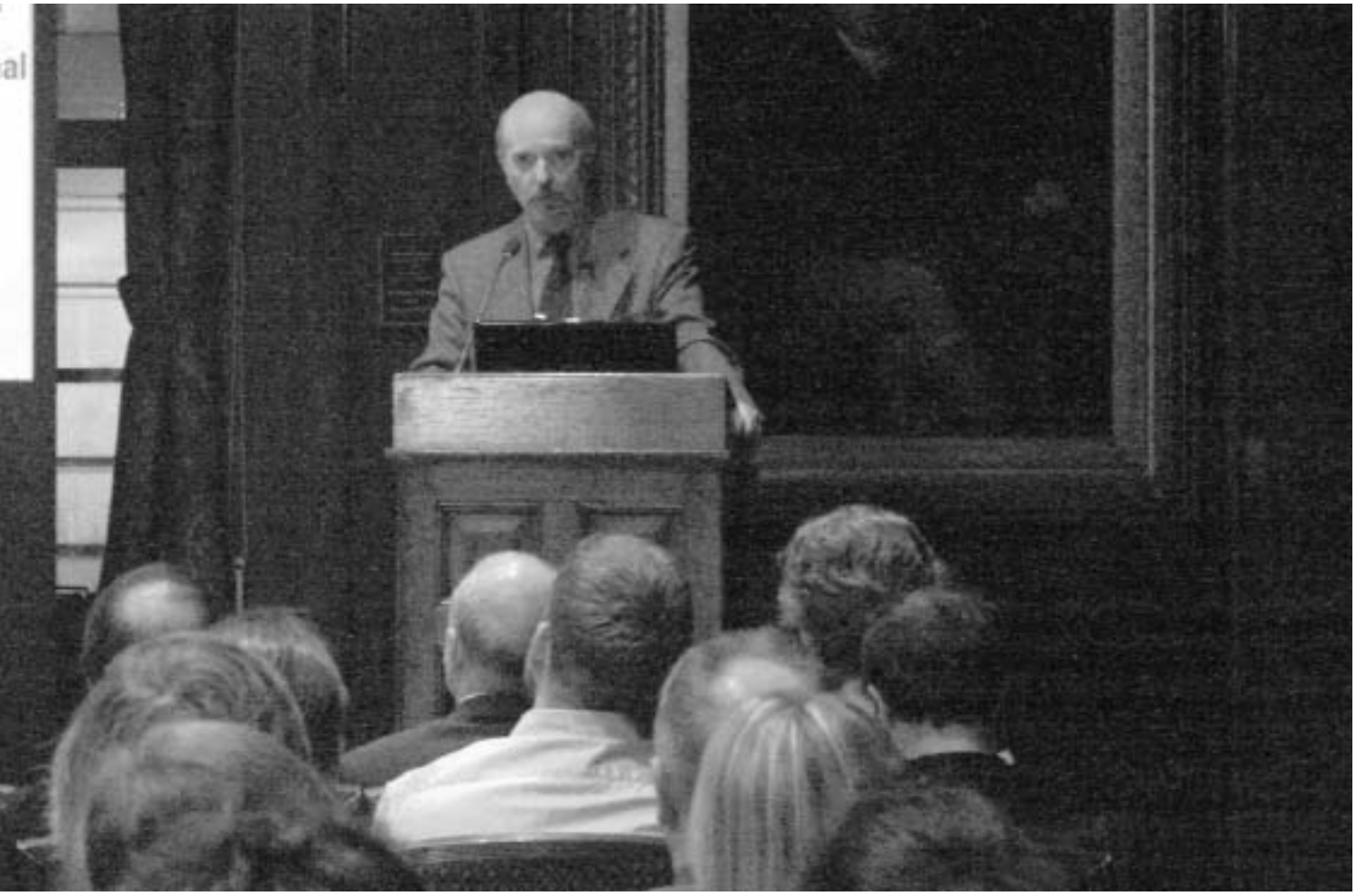
To start with, we set about 'domesticating' *Ps. putida*, says Victor de Lorenzo. This means taking out any bacteriophage sequences from the genome, and simplifying both the physical structure and metabolism by removing flagellar proteins.

Then we need to consider whether *Ps. putida* is suited to the environments where we want to use it. Metabolically it's geared towards reductive power, producing a high proportion of NAD(P)H and there is no standard glycolysis pathway. So, how to get over the problem of anaerobic growth? The answer: think like an engineer.

Firstly, *Ps. putida* has a partial acetate synthesis pathway, which is via a phosphotransacetylase enzyme. This is a good place to start, says Victor de Lorenzo, but we have to turn to other organisms for suitable approaches to generate ATP and regenerate NAD+. Ultimately, we end up with a synthetic operon containing genes for an *E. coli* acetate kinase, and pyruvate decarboxylase and alcohol dehydrogenase from *Zimomonas mobilis* (used in the production of fermented drinks in Mexico). Acetyl-P is converted to acetate with the production of ATP from ADP; pyruvate is converted to acetaldehyde and then ethanol, with the regeneration of NAD+; and *Ps. putida* will now grow under the desired anaerobic conditions.

Additional genetic elements are introduced to prove that, in principle, *Ps. putida* can degrade a specific environmental pollutant — 1,3-dichloropropene. It works, and the pollutant is degraded but the bacteria do not grow well. Perhaps there is a problem with having all these activities in one organism?

Once again, an engineering approach comes in. Maybe we can create a consortium of individuals that contribute different elements of the ability to break down an environmental pollutant? Great, but what if the bacteria don't naturally



associate? No problem, we just stick them together!

Following proof of principle experiments that used a transmembrane domain from *Neisseria* IgA, in combination with leucine zippers, Victor de Lorenzo has developed a system that can make associations between bacterial cells with a given stoichiometry and architecture. Using camel antibodies and other cell membrane proteins, he has created specific associations that occur at either the poles or the middle of an individual bacterium.

So, we see the basis, now, of a modular bacterial system that can be built together, in three dimensions, to produce a desired activity that breaks down environmental pollutants. The final hurdle is to ensure that members of each built consortium are able to communicate.

Victor de Lorenzo has tested a system of Boolean logic gates based on knowledge of chemical signals that are secreted or presented at the cell surface and can dictate the action of transcription factors in another cell. It is possible, he says, to go through the entire literature on transcription factors and translate it into a system of logic gates. He gives an example of what he calls the 'logicome' of the TOL plasmid of *Ps. putida*. Elements of this logicome are selected to engineer communication between individual bacteria and tested using a light-emitting reporter gene. This aspect of the work is ongoing.

All together this is a fascinating endeavour, with the potential to create a powerful toolkit. Victor de Lorenzo adds that to do this on a grand scale, the toolkit must be standardized around:

- Function-bearing implants.
- Genetic and genomic tools.
- Destination chassis.

To this end, there is an aim to create a Standard European Vector Architecture. This is an open access vector collection with universal standards and nomenclature.

watch **online**

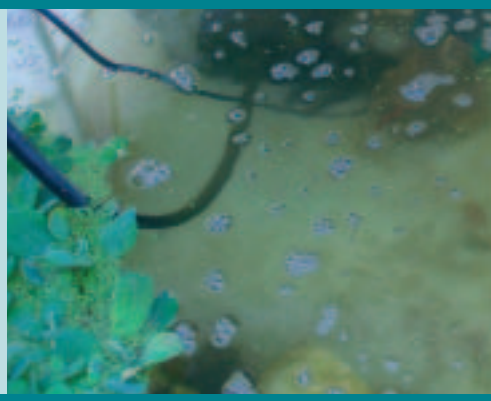
The 2013 *Sf*AM *Environmental Microbiology* lecture is available to watch online at **bit.ly/EMI_Lecture_2013**

Nancy Mendoza

Tuesday 1 – Wednesday 2 April 2014

Activated Sludge Meeting

Control of waterborne disease: a century of the activated sludge sewage treatment process



The Lancashire County Cricket Club, Manchester, UK

Programme*

*Please note that this is a provisional programme and likely to change. For the latest information please visit www.sfam.org.uk/en/events/index.cfm/activatedsludge

Tuesday 1 April 2014

13.00 – 14.00 **Tea, coffee and registration**

Session 1: History and microbiology

Chair: **Ann Soares,** Cranfield University, UK

14.00 – 14.30 **Introduction: development, success and future of the activated sludge process**
Mike Dempsey, Manchester Metropolitan University, UK

14.30 – 15.00 **Faecal indicators of sewage pollution**
Dave Kay, Aberystwyth University, UK

15.00 – 15.30 **Activated sludge: diversity, dynamics and design of the microbial community**
Tom Curtis, Newcastle University, UK

15.30 – 16.00 **Tea and coffee**

16.00 – 16.30 **Metaproteomics for a functional insight to activated sludge**
Paul Wilmes, University of Luxembourg, Luxembourg

16.30 – 17.00 **From bulking sludge to granular sludge**
Mark van Loosdrecht, TU Delft, The Netherlands

Wednesday 2 April 2014

08.30 – 09.00 **Tea, coffee and registration**

Session 2: Hazardous chemicals removal (protecting surface water for potable use)

Chair: **Thomas Curtis,** Newcastle University, UK

09.00 – 09.40 **Effects of pharmaceutical compounds on selection for antibiotic resistance in aquatic microbes**
William Gaze, University of Exeter Medical School, Exeter, UK

09.40 – 10.20 **Cytotoxic drugs, endocrine disruptors, and illegal drugs: where do they go? (Sex and drugs and rock 'n roll: where will it end up?)**
Mark Scrimshaw, Brunel University, London, UK

10.20 – 10.50 **Tea and coffee**

To register online for this meeting please visit www.sfam.org.uk/en/events/index.cfm/activatedsludge

10.50 – 11.30 **Pharmaceuticals, chiral drugs, illicit drugs and sewage epidemiology**
Barbara Kasprzyk-Hordern, University of Bath, UK

11.30 – 12.10 **Removal of hazardous chemicals during activated sludge treatment**
Elise Cartmell, Cranfield University, UK

12.10 – 13.10 **Lunch**

Session 3: Nitrification

Chair: **Mike Dempsey,** Manchester Metropolitan University, UK

13.10 – 13.50 **Relationships between ammonia and nitrite oxidizing bacteria in activated sludge: guild ecology and chaotic instability**
David Graham, Newcastle University, UK

13.50 – 14.30 **Nitrous oxide as early warning of nitrification failure in activated sludge**
Tom Stevenson, Cranfield University, UK

14.30 – 15.00 **Tea and coffee**

Session 4: Phosphate recovery

Chair: **To be confirmed**

15.00 – 15.40 **Omics approaches to enhanced biological phosphate removal**
Per Halkjaer Nielson, Aalborg University, Denmark

Session 5: Gene transfer

Chair: **To be confirmed**

15.40 – 16.20 **Metagenomic approaches to horizontal gene transfer in activated sludge via phage**
Leonid Kulakov, The Queen's University, Belfast, UK

Session 6: Epilogue

Chair: **To be confirmed**

16.20 – 17.00 **Back to the future: full-flow anaerobic wastewater treatment**
Ana Soares, Cranfield University, UK

17.00 **Meeting ends**

Or contact Sally Hawkes ■ Email: sally@sfam.org.uk ■ Telephone +44 (0)1933 382191

30 June – 3 July 2014

Summer Conference

Zoonoses

- In conjunction with the Med-Vet-Net Association
- **Including the *Journal of Applied Microbiology* (JAM) Lecture**

The Grand, Brighton, UK

Programme*

Monday 30 June 2014

11.00 – 17.00 **Workshop session**
International zoonoses collaboration

18.00 – 19.00 ***Journal of Applied Microbiology* lecture: bacterial metabolism in the large intestine, and its consequences for the host**
George Macfarlane, University of Dundee, UK

19.00 – 20.00 **Drinks reception and buffet**

20.30 onwards **Quiz night**

Tuesday 1 July 2014

Session 1: Risk research
Chair: To be confirmed

09.00 – 09.35 **The influence of acquired immunity on the risk assessment of *Campylobacter***
Arie Havelaar, National Institute for Public Health and the Environment and University of Utrecht, The Netherlands

09.35 – 10.10 **Uncovering the real burden of zoonoses**
Tine Hald, Technical University of Denmark, Denmark

10.10 – 11.05 **Tea, coffee and trade show**

11.05 – 11.40 **Modelling the species jump: assessing the risk of zoonotic influenza infection**
Andrew Hill, Animal Health and Veterinary Laboratories Agency, UK

11.40 – 12.15 ***E. coli* source attribution**
Annemarie Käsbohrer, National Reference Laboratory for Antimicrobial Resistance, Germany

12.15 – 13.15 **Lunch and trade show**

Session 2: Host pathogen interactions
Chair: Roberto La Ragione, President Med-Vet-Net Association

13.15 – 13.50 **The virome in health and disease**
Jonathan Heeney, University of Cambridge, UK

13.50 – 14.25 **Alternative models for the assessment of virulence**
Rick Titball, University of Exeter, UK

14.25 – 14.45 **Tea, coffee and trade show**

14.45 – 15.20 **Colonization of MRSA on porcine nasal mucosa**
Birgitta Duim, University of Utrecht, The Netherlands

15.20 – 15.55 **Host pathogen interactions in *Salmonella***
Paulo Pasquali, Istituto Superiore di Sanità, Rome, Italy

16.00 – 17.00 **Attended poster session**

17.00 – 18.00 **Student session**

17.00 – 19.30 **Trade show with wine, buffet and a competition**

Wednesday 2 July 2014

Session 3: Epidemiology and surveillance
Chair: To be confirmed

09.00 – 09.35 **Policy and surveillance constraints on developing efficient surveillance strategies**
Katharina Staerk, Royal Veterinary College, London, UK

To register online for this meeting please visit www.sfam.org.uk/summer

*Please note that this is a provisional programme and likely to change. For the latest information please visit www.sfam.org.uk/en/events/index.cfm/summer_conference

09.35 – 10.10 **Seroepidemiology in foodborne infections**
Kåre Mølbak, Statens Serum Institut, Copenhagen, Denmark

10.10 – 10.45 **Surveillance based on whole genome sequence data**
Marion Koopmans, National Institute of Public Health and the Environment, The Netherlands

10.45 – 11.05 **Tea, coffee and posters**

11.05 – 11.40 **Preventing ESBLs in the food chain**
Dik Mevius, CVI Lelystad, The Netherlands

11.40 – 12.15 **Antibiotic resistance in *Salmonella*: from phenotype to genotype and back again**
Laura Piddock, University of Birmingham, UK

12.15 – 12.50 **Metagenomics for investigating niche adaptation in the food chain**
Sam Sheppard, University of Swansea, UK

12.50 – 13.50 **Lunch and networking**

13.50 – 15.30 **SfAM and Med-Vet-Net Association Student Members' oral presentations**

15.30 – 16.30 **Attended poster session**

16.30 – 16.35 **Introduction to the New Lecturer Research Grant**
Martin Adams, President of SfAM

16.35 – 17.10 **SfAM New Lecturers Research Grant Lecture**
To be confirmed

17.10 – 17.15 **Introduction to the W H Pierce Prize**
Martin Adams, President of SfAM

17.15 – 17.50 **W H Pierce Prize Lecture**
To be confirmed

17.50 – 18.20 **SfAM Annual General Meeting**

19.00 onwards **Drinks reception and conference dinner**

Thursday 3 July 2014

Session 4: Detection and control of neglected and emerging zoonoses

Chair: **Christine Dodd, University of Nottingham, UK**

09.00 – 09.35 **Detection of new and emerging zoonotic viruses (including foodborne)**
Wim van der Poel, Central Veterinary Institute of Wageningen University, The Netherlands

09.35 – 10.10 **New coronaviruses, common genetics and pathobiological features**
Astrid Vabret, University Hospital of Caen, France

10.10 – 10.45 **Tea, coffee and posters**

10.45 – 11.20 **Factors driving the epidemiology and control of Crimean–Congo haemorrhagic fever (CCHF) in Europe**
Agustin Estrada, University of Zaragoza, Spain

11.20 – 11.55 **Advances in diagnosis of lyssavirus/ advances in prevention of rabies**
Tony Fooks, Animal Health and Veterinary Laboratories Agency, UK

12.15 – 13.15 **Lunch and close**

Or contact Sally Hawkes ■ Email: sally@sfam.org.uk ■ Telephone +44 (0)1933 382191

membership options

■ **Full Ordinary Membership** gives access to our many grants and awards, online access to the *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*, 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.

■ **Retired Membership** is available to Full Members once they have retired from their employment. Retired Members are entitled to all the benefits of Full Membership except grants and access to the Society's journals.

■ **eAffiliate Membership:** This category of membership is open to microbiologists residing in Band I developing countries and is free of charge. It is an online only membership and provides access to the eAffiliate bursary only.

■ **eStudent Membership:** This category of membership is open to undergraduate students only. It is an online only membership and is free of charge. This category of membership does not provide access to the Society's grants or 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*.
- 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).

JOIN US!

You can apply for membership on, or offline. To apply offline, please contact the Membership & Finance Co-ordinator, Julie Wright on **+44 (0)1234 326846**, or email **julie@sfam.org.uk.**

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:

- The opportunity to apply for one of our many grants or funds.
- Eligibility to win any of our awards or nominate a candidate for the SfAM Communications Award.
- Access to our five peer–reviewed Journals: *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*.
- Free access to the entire collection of digitized back files for *JAM* and *LAM* dating back to 1938.
- A topical quarterly magazine, *Microbiologist*.
- Substantially reduced rates for attendance at SfAM meetings and conferences.
- Networking with worldwide professionals in over 80 countries.
- Access to private members' area of the SfAM website.
- Monthly email bulletins with the latest news from SfAM.
- Invitation to the annual *Environmental Microbiology* lecture.
- Fostering cross disciplinary research.
- A 25% discount on the extensive Wiley–Blackwell collection of titles.

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 Lecture Grants 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 application forms.

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 Wiley–Blackwell in the monthly journals: *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*.

All Full and Student Members receive free access to the online versions of the Society's journals, and can also submit papers to our journals via an online submission service.

MEETINGS: We hold three annual meetings; the Winter Meeting is a one-day meeting with parallel sessions on topical subjects. The Spring Meeting is a one–day meeting tailored for personnel in clinical microbiology. The Summer Conference is held every June/July and comprises a main symposium, a poster session, the AGM and a lively social programme. All members are invited to our prestigious annual lecture held to commemorate the success of our *Environmental Microbiology* journal. We also hold joint ventures with other organizations on topics of mutual interest.

WEBSITE: The website is the best source of detailed information on the Society and its many activities. It has fully interactive membership areas where you can find archive issues of *Microbiologist*, exclusive SfAM documentation and much more.

Membership changes

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

M. Hawley; A. Hawley

Canada

C. Belanger

Denmark

L. Rickelt

Ethiopia

B. Teshome

India

A. Gupta

Ireland

C. Dalton; C. Healy; S. Mok; T. Sojinrin; Y. Tan

Mexico

L. Espinosa; D. Martinez; D. Rivera

Nigeria

M. Adebayo; S. A. Akinola; C. A. Anyiam; A. E. Ojewole; C. Ubadire-Agua

Portugal

C. Amorin

Uganda

J. H. Musalima

UK

B. Hunt; J. Akhtar; S. Ali; M. Aljubouri; Y. Amereen; I. A. Andrioae; A. Ather; C. Awofeso; N. Bhattarai; J. Bird; K. Bobrik; C. Pin Bong; L. Brown; G. S. Bulmer; O. Butt; W. Carr; M. Carter; A. Chan; M. Chan; S. Choudhry; M. Christodoulou; A. Christopher; T. W. Convery; S. Coppack; A. Crookes; A. Dahir; O. Day-West; L. Delaney; R. Draheim; L. Eshmene; F. Fajandar; F. Fitzgerald; R. E. Folds; K. Giannoutsos; C. Gibson; N. Gibson; A. Gillum; L. Godden; B. L. Greenwood; J. Gutierrez-Merino; S. Hang; B. Harman; F. Harrison; A. Hopkins; H. Huckson; P. Hutchinson; R. Jeffcock; T. Jeyapalan; N. Jimmy; F. Kartal; K. Kauser; L. Kelly; N. Khumalo; J. Kirk; R. Kuzminas; A. Lazar; G. L. Liddle; G. Lock; M. Lukumbuzya; S. Marcelin-Home; H. Mazi; K. McDade; K. McKay; L. Mehigan; R. Mohan; J.-M. Moser; G. Nabh; H. Naqvi; R. Nayak; F. L. Noyce; M. A. Pallett; K. M. Parfitt; J. C. Parker; M. S. Patel; D. Patel; D. J. Payne; R. Phillips; J. Pietrzyk; L. Plant; D. Premkumar; F. Rashid; E. Rogerson; Z. Ross; G. Rowley; K. Ryan; S. Saleem; M. Saleh; L. Scanlon; L. Scardarella; D. Sharp; C. Shaw; B. A. Shearman; E. A. Slade; J. E. R. Small; J. Smalley; K. Smith; K. S. Jayan; S. Stanney; P. Stevenson-Leggett; M. Taubert; L. Taylor; L. M. Terry; S. Thapa; L. Thiyagarajah; T. Thompson; K. S. Watts; D. Wilkinson; T. C. Wilson; M. N. Zahid; M. E. J. Zalm

USA

K. Bauer; R. L. Jeffery; L. Laurens; R. Neve; A. Quashie; S. Simeonidis

Losses

We were saddened to learn of the death of the following Members of the Society:
Brian Dancer, Louise Fielding and Fred Skinner.

Call for nominations for W H Pierce Prize

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 now worth £3000.

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 2014 prize should write in confidence to the Honorary General Secretary, Professor Mark Fielder, at the Society Office in Bedford, including a full CV of the nominee and a letter of support. Please note that application is through nomination by Full Members of SfAM only and that there are no official forms for this award.

Closing date for nominations is Thursday 17 April 2014.

S*f*AM past President honoured by Science Council

Margaret Patterson (S*f*AM President 2005–2008) has been named among the UK's 100 leading practising scientists.

In a move designed to counter what the Science Council described as the "*UK's narrow and old-fashioned view of science*", they have compiled a list of scientists who are actively involved in generating knowledge; teaching and mentoring; contributing to policy formation and communicating with lay audiences; ensuring the translation of research into business endeavours; working to make the application of science safe and legal; and delivering services to support modern life.

Margaret Patterson has been recognized for her expert leadership in the regulation of research into high-pressure processing of foods and food irradiation. Margaret's current roles are as Senior Principle Scientific Officer, Sustainable Agri-Food Sciences Division of the Agri-Food and Biosciences Institute in Northern Ireland; Honorary Reader in the School of Biological Sciences, Queen's University, Belfast; and Fellow and current President of the Institute of Food Science and Technology. She is also a Chartered Scientist.

Also on the list is Colin Dennis, the 2014 S*f*AM Denver Russell Memorial Lecturer and Chairman of the English Food and Drink Alliance.

The full list of 100 leading UK practising scientists is available here: **bit.ly/SC_100.**

Nancy Mendoza

OBITUARIES

Frederick (Fred) Arthur Skinner (1919–2013)

To walk through a garden with Fred Skinner on a sunny summer's day was the pleasure of being with a gentle lover of plants and flowers. Gentleness characterized him and his life. Fred was born in London and attended Battersea Grammar School, where he excelled as a pianist, and from which in 1939 he was awarded a scholarship to Emmanuel College, Cambridge to read Natural Sciences. His love of plants was probably acquired while reading botany during two periods as an undergraduate, separated by six years of army service from 1940 to 1946. This was principally in the Royal Electrical and Mechanical Engineers, where he taught physics, electronics and the principles of radar, and from 1943 to 1945 he served in Algeria and in Italy. After demobilization, Fred joined the staff of the Rothamsted Experimental Station in Harpenden, where he worked for the whole of his professional life. In 1953 he was awarded a PhD from the University of London. In 1957 he became a Member of the Institute of Biology and in 1973 he was made a Fellow.

Fred's interests in soil bacteriology began with a paper in *Nature* about actinomycetes and this interest persisted until in the early 1960s, in a co-operation with Jack Pepys at the Brompton Hospital, the cause of Farmer's Lung was discovered to be an actinomycete. In 1968, an interest in *Rhizobium* (nitrogen-fixing soil bacteria) emerged and continued until Fred's retirement in 1980. Characterizing Fred's interests, notably his first paper, were frequent publications on methods, culminating in his distinguished tenure from 1966−1992 as Editor of the Society's *Technical Series*. For Fred, scientific enquiry never ended; in his last years he was interested in attempting to germinate fern spores on agar media.

Fred Skinner's distinguished service as an Editor of Society publications began in 1965 and continued for a remarkable 26 years. During this time he was Editor of the *Journal of Applied Bacteriology*, the *Technical Series* and the *Symposium Series*. Authors, particularly jealous or opinionated ones, can be difficult to deal with but Fred was always calm. Many, including me, began their editorial life under his gentle guidance and long-lasting friendships resulted. Early during his military service Fred found himself manning a searchlight on the Isle of Grain in the

Thames Estuary. He could also shine a clear light on editorial problems and simplify complicated syntax with enviable ease. When his editorial activities ended he served as President from 1991 to 1993. Fred received a special award from S*f*AM in recognition of his exceptionally long membership of over 60 years, the only time such an award has been made.

Fred Skinner enjoyed the social life of Rothamsted and in its Tennis Club he met Mary, his wife to be, in 1948. He joined in enthusiastically with the social activities that are so central to the life of our Society, and he attended Summer Conferences throughout his life and Mary would organize entertainment for accompanying wives. Fred was easy-going and to be near him was never to be far from laughter.

Fred's three sons and their families were central to his life, and he was immensely proud of them and his grandchildren. In his latter days Fred was confined to a nursing home, where he was loyally supported by members of the Harpenden United Reformed Church, of which he had long been an active member, and by the daily visits of his wife Mary. Fred Skinner touched many lives in many ways and he will be greatly missed.

Max Sussman

Professor Louise Fielding (1968–2013)

Louise Fielding, a highly valued member of the S*f*AM Executive Committee from 2007 to 2013, passed away peacefully in December.

She was an active member of the S*f*AM Meetings Subcommittee, contributing to the ongoing success of the annual Winter Meetings, Spring Meetings and Summer Conferences. Amongst other contributions, she organized a Risk Assessment workshop at the 2011 Summer Conference and had been due to chair a Food Contamination session at the January 2014 Winter Meeting which she had also organized. At Summer Conferences, Student Members were notably encouraged by the interest Louise used to take in their poster presentations.

The production of *Microbiologist* changed significantly in 2008 with the formation of the S*f*AM Publications Subcommittee, recently renamed as the *Microbiologist* Editorial Group. Louise was one of the original members of this group as a Features Editor. In this role Louise commissioned and edited articles for the quarterly members' magazine. Her knowledge and enthusiasm were both great assets which resulted in the publication of many interesting articles.

Louise studied at the University of Reading, graduating with a Bachelor's degree in Food Technology (1990) and PhD in Food Microbiology (1995). Her subsequent career, spanning more than 20 years, was based at Cardiff Metropolitan University, formerly known as the University of Wales Institute, Cardiff (UWIC). Her principle research interests were related to the implementation of food safety management systems and novel methods for decontamination of bioaerosols and surfaces. She had a unique facility for working with ozone and also investigated *Listeria monocytogenes* infections associated with elderly residents of care homes. She had been the Director of Research in the School of Health Sciences since 2009 and was awarded a Personal Chair of Food Safety in August 2013.

Although based in Wales, Louise was involved in food safety on an international level too, being a member of the International Association for Food Protection and recently undertaking a consultancy for the Food and Agriculture Organization of the United Nations in Bangladesh.

All S*f*AM Members and associates who knew Louise will have their own personal memories of her but there are some common threads which run through the messages received since her untimely death. Louise is remembered most for her good-humoured positive outlook, enthusiasm for sharing knowledge and refusal to let setbacks become obstacles. Her love of shoes is particularly prominent in many minds!

How better to summarize Louise's life than with the words of those who knew her:

- "Exemplary in her devotion to her students."
- "Very welcoming and a lot of fun too."
- "Much more than just a fellow committee member — she shared her knowledge and experience and was a kind of mentor."
- "An inspiration by her ability to maintain her smile, energy and enthusiasm."
- "Infectiously warm, a friendly and ever-helpful person."
- "Brought a sense of fun combined with professionalism in everything she did."
- "A great travel companion."
- "Combined knowledge, subject passion and humour in a unique way — Lou will be greatly missed."

The Society is thankful for Louise's many contributions and extends its heartfelt condolences to her family and friends.

Louise Hill-King

In the loop pecs

News from the S*f*AM Postgraduate and Early Career Scientist Committee

Public engagement workshop — S*f*AM Summer Conference 2013

The public engagement workshop was the unofficial start of the S*f*AM Summer Conference 2013 in Cardiff.

S*f*AM's Communications Manager, Nancy Mendoza welcomed everyone and introduced the first speaker of the day, Steve Cross. Steve is Head of Public Engagement (PE) at UCL and, amongst other activities, also runs the well-known Science Showoff.

Steve introduced himself and asked the audience if they had been involved in any PE activities in the past. The audience responded vibrantly as many of those present had been previously involved in science festivals, STEM activities such as 'Lab in the Lorry', volunteering for S*f*AM at PE activities, along with many other examples.

Steve explained the definition of PE, according to UCL, as "*talking, listening and being prepared to change, offering benefit to everyone involved*". PE involves informing, inspiring, consulting and collaborating to solve a problem. Good PE activities generate a mutual benefit with all parties learning from each other through sharing knowledge, expertise and skills. The whole process should 'build trust, understanding and collaboration, and increase the sector's relevance to, and impact on, civil society'. PE activity should change researchers' and audience's awareness, attitudes, skills and power.

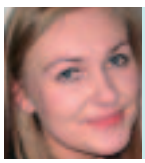
However, the most important thing is to think about whom you are directing your activity towards. It cannot be just 'the public' as it has to be more specific and thought through. You need to define: demographics, age, shared interest, location, needs, community, etc.; you also need to identify, why you want to engage. Most researchers do it for personal, moral, academic or business reasons. Steve highlighted some great benefits that PE activities can bring for those organizing it. You can gain skills development, career enhancement, new research ideas, a higher personal and institutional profile, and even potential new collaborations. So, do it because it is fun, remember it takes more time than money, and enjoy the benefits.

The next speaker of the morning session was Jo Verran, Professor of Microbiology at Manchester Metropolitan University. Jo's research interests are the attachment of microorganisms and biofilm formation. She also has an interest in combining microbiology and art.

Jo's approach with PE is to identify areas of research that contain relevant and important messages, which can be delivered to an audience in a relatable and understandable form. Again, Jo highlighted the need to consider who your audience is and how to get their attention. Jo listed a number of examples of PE activities. There are many options and opportunities such as local science festivals, big music festivals or school outreach activities.

Jo reminded us you don't always have to organize everything from scratch; you could just help someone and contribute some of your ideas. There are plenty of people and organizations who will undertake PE with you, such as your supervisor, your colleagues, people from your own institution or other universities and professional bodies, such as S*f*AM.

Jo's strategy is to amaze her audience by using popular topics, and combining art and science. Jo has organized and taken part in various activities, such as 'The Good, the Bad and the Algae' — to show how much algae there is in a drop of water; 'Plaque Attack' — all about biofilms on teeth; 'Handwashing Activities'; and the 'Bad Bugs Book Club'. Jo explained how it was possible to link the Twilight series to microbiology, as well as other examples from popular culture. Finally, Jo encouraged the audience to get involved in PE, saying that it can have a really positive impact on your future research and your personal development.



Agnieszka Piotrowska

After lunch, the afternoon session began with Jayesh Shah, from Ipsos Mori, giving us an interesting talk on statistics relating to public engagement (PE), whilst telling us how to engage correctly with different groups for the best effect. Jayesh's graph showing that scientists are amongst the most trusted professionals, unlike estate agents, politicians and other professions, sent laughter through the aisles.

Jayesh began by asking who in the room had been to a museum, art gallery or festival. From these results Jayesh concluded that as a group we were not 'the norm', or average. Jayesh went on to explain, in detail, the different categories of 'the public' and how we can use this information to tailor our events. These categories included confident engagers, distrustful engagers, late adopters, the concerned, the indifferent and disengaged sceptics.

It was extremely interesting to see how you might engage with each of these groups. We were encouraged to dispel myths about science and to understand that different groups of people need to be engaged with in different ways. Jayesh finished by saying that we should be creative and should try to fit in with the interests and daily lives of those we seek to engage.

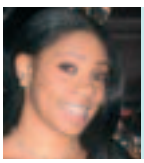
Rhys Phillips, from the Cardiff Science Festival, presented a talk entitled '*Science festivals: what organizers are looking for*?' Rhys described a science festival as an event celebrating and promoting science, engineering and technology, by creating a fusion of the worlds of the sciences and arts. Simply, he described it as the style of a musical festival but with science content. The type of audience, programme, how to make it successful and finances were all discussed. Rhys discussed his own experiences as well as showing attendees how we could all do something similar.

Suzanne Spicer, from the University of Manchester, gave a fascinating talk about evaluating PE. By the end of her talk we really understood how important evaluating a PE event was, and the benefits and challenges of evaluation in this scenario were shown. Suzanne explained how to build evaluation into an activity and ensure that it is designed to answer your questions. It became clear that evaluation is not a bolt-on and needs to be considered when you are planning your activity. We were encouraged to share information so we can learn from each other and put our evaluation to some sort of use. Suzanne finished with her top tip for evaluation which was to be the BOSS, Brief, Objective, Specific and Simple.

For the final session of the day, the panel members sat at five different tables and delegates were given the opportunity to choose who they wanted to sit down and talk with. The topic for our table was '*my microbiology message is...*'. Everyone had an opportunity to move tables every 10 minutes, but most people were too involved in their discussions and stayed firmly where they were. In our group we came up with talking about 'good bacteria'. We didn't end up with one final idea, but we discussed in detail how important it was to look at your audience, and what message you want whilst presenting it in an interesting and fun way.

Each guest speaker gave a brief summary on what their group discussed: 'antibiotics and the worried person', 'creating awareness on communicable diseases' and 'microorganisms on the good side — probiotics'. The group work showed the diversity of potential PE projects and it was a nice close to the session.

The day was brought to a close with a top tip from each of the speakers: know your audience, know why you're doing that activity, get help, be creative and evaluate. It was a wonderful workshop that clearly encouraged people to either get involved or to organize their own event.



Sabrina Roberts

careers

Thirty seconds for a life-changing decision

Jean-Yves Maillard describes his eventful career journey

Eighteen years ago, if someone had told me that I would be working and living in the UK, I would have laughed. I was not good at languages and my English was non-existent — although a few colleagues from S*f*AM would probably say that this is still the case today! I resembled the policeman in *'Allo 'Allo*; actually his "*good moaning*" was much better than mine at the time. Worst of all, Britain was a land where the inhabitants boiled meat and drank warm beer. I later discovered that this *cliché* was incomplete as, in addition to boiled meat and warm beer, sandwiches are the national favourite for the daily five-minute lunch.

I am often asked how I ended up

Attending the first SfAM Summer Conference on 'Antibiotic and Biocide Resistance in Bacteria' held in Swansea from 9 – 12 July 2001

Receiving the AD Russell Memorial lecture commemoration prize from Martin Adams (SfAM President) (January 2012). A very happy and honouring moment to give this lecture in memory of my former supervisor and mentor.

living in the UK for so long, and taking a career path leading to a Professorship in Pharmaceutical Microbiology in a UK university. The answer is simple: a split-second decision.

I started my career with a 'Diplome Universitaire de Technologie' in plant sciences, at the University of Lyon in France. This was a two-year diploma with the aim of getting a technician job at the end of it. Success and employment rates for graduates of this course were extremely good, and at the time I did not see myself in higher education for too long. Near the end of the course, the prospect of working was actually not too appealing (I was 20) and when the director of study asked, in passing in the corridor, for volunteers to spend 10 months in a UK university laboratory, I took the decision to go… in 30 seconds. Eighteen years on, I have never looked back.

My early career path was slightly unconventional. After 10 months in a microbiology research laboratory at the University of Wales College, Cardiff, with very patient co-workers (how many times can one repeat "*Would you like a cup of tea*?" before one understands?), my supervisors, J. Fry and M. Day, suggested I join the BSc in Microbiology and Genetics, which I did. Two years later, I graduated with honours and was very fortunate to be introduced to Denver Russell who offered me a PhD looking at the interactions of biocides with viruses. Denver was an excellent supervisor and, little known to me at the time, a world-renowned expert on biocides. I completed my PhD in under three years and was examined by Stephen Denyer.

Such details in my early career might seem superfluous, but both Denver Russell and Stephen Denyer had a huge impact on what was to come. Denver was a world-recognized giant in biocide research, a niche subject of great importance which I have since embraced fully. Stephen Denyer was at the time convenor of S*f*AM's Pharmacy Interest Group.

I joined S*f*AM in 1991 and enjoyed the conferences (especially as a student — there were many late nights at S*f*AM Summer Conferences!). Following my PhD, and after a 12-month spell in the French Army as a commissioned officer in the Health Corp, I started a lectureship in Pharmaceutical Microbiology at Cardiff University, with Denver Russell as a mentor. At the same time, Stephen Denyer proposed me to S*f*AM as convenor of its Pharmacy Interest Group. These two events shaped what I am doing today.

Through academic progression and a really enjoyable four-year spell at the University of Brighton, I progressed through the academic ranks.

From being an interest group convenor, I became an S*f*AM Executive Committee Member and the Chief Editor of *Letters in Applied Microbiology*.

All these S*f*AM experiences, especially as a junior academic, were extremely useful and I would recommend them to anyone. The convenor responsibility was particularly interesting; the objective of the interest group was to organize scientific meetings for the Society. I inherited a group of much more experienced scientists, some quite senior, that I had to engage with to get ideas for meetings and put meeting programmes together. This was an ideal opportunity to gain experience rapidly and to network. This culminated in the first S*f*AM conference on biocides which took place in Swansea in 2001. This was a brilliant conference, both scientifically and socially (some attendees still speak of it today!). The opportunity of joining S*f*AM's Executive Committee at a time of many changes within the Society was a fantastic one (thanks to Arthur Gilmour — he is probably still cursing buying me that drink at the Swan Hotel in Bedford). By this time you will have noticed that the word *opportunity* is quite central here — and with the drive from Arthur (who will probably still deny it), S*f*AM became the vibrant and dynamic learned Society that we have today.

So what is a typical day for me and what are my responsibilities? My wife would certainly like to know the answer to the first question, as I invariably say "*very busy*" when she asks the daily question "*how was your day*?" The truth is I do not have typical days. I am

involved in so many activities that no two days are the same. After almost 20 years, I have yet to have one boring day at work; some will say that this is lucky and indeed I really like my job (or jobs, to be more accurate), although sometimes I wish it would be slightly less busy. Currently I have a research group comprising of four PhD students (two writing up), four postdoctoral fellows and one research technician. Although my researchers are fairly independent, research demands planning, discussion of results, more planning, reports, and reading of scientific papers and other documents. My role also involves writing peer-reviewed papers, reviews, book chapters, books, and peer-reviewing scientific articles, reviews and grant applications for funding bodies.

Working on biocides, a large aspect of my work is closely linked to industry, which demands even more reports and some travelling. I also work as a consultant for the industry via the university. This certainly keeps my technician busy; at one point last year, she was working on six different projects.

I am also working with the European Union as an expert on biocide resistance, and this work in particular demands a lot of reading and writing. It is, however, very satisfying to see that my contributions to EC documents such as the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and the Scientific Committee on Consumer Safety (SCCS) are widely quoted and referenced by the industry, as well as academics too. These documents contributed to the Biocidal Product Regulation, a regulation that is key for the commercialization of biocidal products in Europe.

One important aspect of academic life is securing research funding. Unfortunately, the field of biocides has always sat between fundamental and applied research, and for some research funding bodies, biocide research is too applied; for others, it is not applied enough. That said, the field of biocide research gained some importance a few years ago with the increased mortality rate in UK hospitals associated with methicillin-resistant *Staphylococcus aureus* and now *Clostridium difficile* outbreaks. These unfortunate events improved public understanding of infection, infection control and hygiene, and focused somewhat the minds of decision-makers on prevention. It also contributed to the explosion in the

With Peter Setlow and Fernanda Mozzi, members of the joint Editorial Board for LAM and JAM at Cardiff Castle (July 2013). A real pleasure to be Chief Editor and meeting really nice Editors

number of consumer products containing biocides (check your groceries). The number of products (consumer, healthcare, etc.) containing biocides, together with recent questions about bacterial resistance to biocides and cross-resistance to unrelated antimicrobials such as antibiotics, are also leading to more research funding opportunities in the field of biocide research (although this remains very competitive). PhD and postdoctoral fellows need to be replaced (or their contracts extended) and that demands funding. So a significant, although less enjoyable, part of my job is to source such funding.

A more pleasurable aspect of my work is being invited to talk at conferences and I travel a fair bit (far too much according to my wife, who takes a strict, possibly accurate, note of my days away from home). I get to meet old and new colleagues at these events (usually over some beers) — over the last two years I have been invited to talk at 18 international meetings (the last one being in Australia's Gold Coast).

As an academic, I am also involved in undergraduate teaching. Unlike *Unseen University* (for Terry Pratchett fans), I recognize students (although very vaguely) and I have to turn up at lectures, practicals and workshops. This usually causes confusion and fear to my research group, as this is the rare occasion when I wear a lab coat; their fear is that I intend to actually do some lab work (which of course I do threaten to do from time to time).

As if I did not have enough to do, I am also Chief Editor of *Letters in Applied Microbiology* (LAM). As this is an international journal, I receive manuscripts from all over the world. Our joint board of Editors (with the *Journal of Applied Microbiology*) is composed of at least 50% overseas Editors. This means I receive manuscripts and reports from Editors 24/7 — a never-ending job. Although it is rather time-consuming, I really enjoy this job. It gives me the opportunity to keep in touch with S*f*AM's Executive Committee, to the great lament of Phil Wheat, its CEO. I am not the quietest invited Member (although I am quieter than Andy Sails — I had to drop his name in this article). I have to be in regular contact with LAM publisher Wiley-Blackwell, with whom S*f*AM has enjoyed many years of a good and profitable relationship.

Finally, add in my duties as an external examiner for undergraduate courses such as BSc (Microbiology), MPharm, (Pharmacy) and for postgraduate courses such as MRes, MPhil and PhD. This involves commenting on exam questions, reviewing the fairness of marks, reading and commenting on Master and PhD theses, and conducting *viva voce* examinations.

So my daily life is all but monotonous, which is probably the reason why I love my job. I have probably forgotten some of my activities, as I am always taking on new challenges. All of this would not have been possible without the unwavering support of my wonderful, long-suffering wife (this sentence was added by my wife post-editing).

So, would I recommend what I do to anyone? Absolutely. Any advice? Don't hesitate to make decisions on the spot and seize any opportunities that may come your way. The world is your oyster. Finishing with a food quote seems appropriate for a Frenchman who lost his way in the *perfide Albion* (a name for Great Britain coined by Victor Hugo, author of *Les Misérables* — he spent some time in Guernsey and was probably thinking about food!). Maybe the S*f*AM CEO can take note that I do miss the three-hour lunch complete with five-course meal!

Jean-Yves Maillard
Cardiff University

Students into Work Grant report

am I eligible — can I apply?

Yes — if you are FULL Member who can offer an undergraduate microbiology student the chance to obtain work experience. If you would like to read about the experiences of students who have benefited from this grant, you can do so below.

For further information visit: **http://www.sfam.org.uk/siw**

Antiseptics and methodology for routine testing

I am a BSc (Hons) Biomedical Science undergraduate student from the Manchester Metropolitan University (MMU). I feel extremely privileged to have been a part of the MMU life; the facilities and staff are fantastic. With the recession, reduction in funding and increased cost of higher education it gets harder and harder to gain work-related experience as a student. Thankfully, Professor Val Edwards-Jones with the support of the Society for Applied Microbiology (S*f*AM) and a Students into Work Grant, offered me a 10 week full-time placement to help me gain microbiology experience in the field of antiseptics and methodology for routine testing.

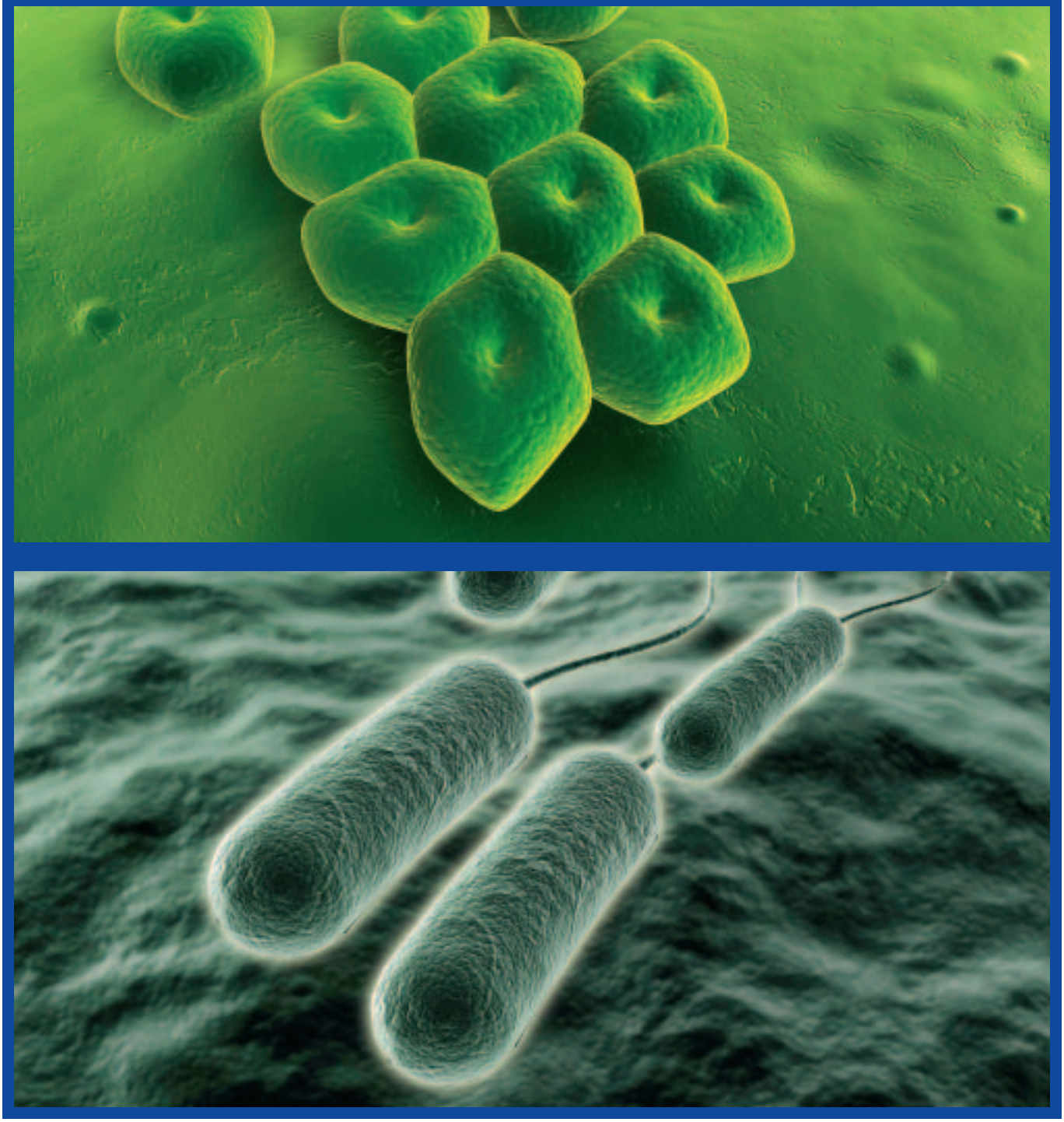
With our increased understanding of the spread of infection, hospitals and other healthcare establishments have greatly improved their cleanliness and infection control procedures. Sadly however, infection from antibiotic-resistant bacteria continues to take so many lives every year. Thus, the need to reduce the use of antibiotics and use alternative approaches to treating certain infections has become a large focus for healthcare companies worldwide. The more often a wound dressing is changed, the higher the chances of acquiring an infection. Therefore, by having more efficient compounds combined with the appropriate wound care technology the lower the chances of infection, which in turn, will reduce the use of antibiotics. Without overuse or incorrect use of antibiotics the increase in antibiotic-resistant bacteria will be reduced and hospital acquired infections with resistant bacteria should also reduce. All around the world research into producing antiseptic wound care has expanded exponentially. Unfortunately, there is still some reluctance of their usage because of some reported side effects and delays in wound healing. Silver dressings have been found to be very effective *in vitro* and via carrier vessels (dressings) however, its clinical efficacy is still questioned by many and in some countries have been removed from their formularies.

The primary aim of the project was to establish routine testing methods for common antiseptics used in wound dressings and test a range of pathogens for susceptibility against them. Routine testing against antiseptics is not normally undertaken in clinical laboratories, and pathogens are tested for susceptibility against a panel of antibiotics and reported appropriately. Thus, on receiving a report from a clinical laboratory, the person attending a patient with a wound (chronic or acute) has little or no guidance on what topical antiseptic to use. The secondary aim was to assess the antiseptics used for synergy or antagonism.

Over the 10 week placement I worked on creating a suitable method to determine the MIC of four antiseptics including silver nitrate, polyhexamethylene biguanide, iodine and chlorhexidine. Once the appropriate solvents were chosen to make various concentrations of antiseptics, the effect of culture media, method used and conditions were all tested to allow a routine testing method to be established. It was found that culture media did affect the MIC but this depended upon the antiseptic used. Eventually after three or four weeks, the method was optimized and the culture medium Müller-Hinton was chosen to continue the work. Agar incorporation was the most efficient method and allowed a number of bacteria to be tested simultaneously; I eventually produced a reproducible method that would allow routine testing of antiseptics that are currently used in wound care. The zone of inhibition method is an easy method to assess susceptibility to antibiotics, but was not particularly reproducible for testing antiseptics. The MICs of the four antiseptics were raised for key microbes such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Figure 1).

I also looked at synergy between the various compounds using the checkerboard method in microtitre trays. I found that two antiseptics

Figure 1. *Acinetobacter baumannii (above) and Pseudomonas aeruginosa (below)*



showed synergy at certain concentrations which were lower than the individual MICs. It is possible, if they were used together in wound care at these lower concentrations, that they would still be antimicrobial but less cytotoxic. This would be an area of further study and unfortunately I did not have time to undertake this.

I would like to give the biggest thanks to the Society for Applied Microbiology for providing me with the funding in order to complete the project. To the students I shared the laboratory with who offered me interesting facts, company and support, to the incredible staff of the MMU Microbiology Laboratory, and to MMU for giving me access to their fantastic facilities and their support throughout my time in higher education; last and most certainly not least, my mentor Professor Valerie Edwards-Jones, for all her help and support throughout the project.

Ashleigh Mitchell
Manchester Metropolitan University

President's Fund report

am I eligible — can I apply?

It is not only our Student Members who require our help. Senior microbiologists often find difficulty in funding attendance at meetings. If you are in this position you are eligible for this fund.

For further information visit:
http://www.sfam.org.uk/presidents-fund

Antibiotic Resistome of the Skin

Commensal bacteria normally live in symbiosis with the host but they are able to switch to a pathogenic lifestyle, once the host is immunocompromised or a new niche (e.g., an indwelling line) is created. *Staphylococcus epidermidis* is an example where some virulent strains can produce biofilms on catheters, and thus are protected from antibiotics and the host's immune system (Otto, 2009). Commensals are tending to acquire more and more antibiotic resistance and some, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, can occur on the skin and may be resistant to the majority of therapeutic antibiotics. For the skin microbiome in particular, the antimicrobial resistome profile has yet to be thoroughly deciphered.

High-throughput technologies have determined the microbiome at different sites of the body; most of the data obtained relates to the gastrointestinal tract rather than the skin. The Human Microbiome Project contributes to this endeavour as 18 skin sites from 250 volunteers have been sampled and sequenced to give an insight into the indigenous microflora. Four different phyla — Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria have been identified with 16S metagenomics. From this group, Actinobacteria is the most prevalent skin bacteria compared with Firmicutes and Bacteroidetes, which are the most prevalent bacteria in the gastrointestinal tract.

Diverse environments on the skin, e.g., sebaceous, moist and dry, account for the differing microbial diversity; this is apparent for certain areas of the human skin where areas producing sebum, such as the face and back, promote growth of lipophilic microorganisms with *Propionibacterium acnes* being dominant. Moist areas, such as the groin, promote the growth of *Staphylococcus aureus*, Gram-negative bacilli and *Corynebacterium* spp. Dry regions such as the leg and arm host fewer bacteria, but this niche has the most bacterial diversity and greater numbers of Gram-negative bacteria, which may be due to transient colonization from the environment (Grice & Segre, 2011).

The occurrence of transient species (which are usually Gram-negative) and shifts from a Gram-positive to a Gram-negative flora are thought to play a part in the aetiology of some diseases; however, research in this area is still ongoing. A shift in the microbiota from Gram-positive to more Gram-negative bacteria has been observed when dressings are applied to the skin, and this has been attributed to an increase in the pH and moisture content.

A number of different factors such as host demographics, genetics, behaviour, environmental characteristics and transmission events all affect the microbial diversity of the skin. Commensals on the skin can protect the host from pathogenic bacteria both directly through competition for nutrients, or by inducing a hostile environment for the pathogen such as the production of bacteriocins e.g., lactobacilli produce nisin which inhibits the growth of staphylococci or indirectly by regulating the innate immune response by producing modulins (e.g., *Staph. epidermidis*), which trigger antimicrobial peptide secretion by the skin cells (Rosenthal *et al.*, 2011).

Whilst microbes have co-evolved with the host, antibiotics can disrupt the beneficial relationship with commensal organisms. There is widespread use of antibiotics for skin disorders such as acne and rosacea, and this selective pressure can last for years (Wilson *et al.*, 2011). Handwashing, the use of lotions, as well as the use of antibacterial soaps also cause fluctuations in the skin microbiota, disrupting the commensals and allowing pathogenic bacteria to proliferate.

Antibiotic resistance genes are ubiquitous in nature and enable bacteria to compete with each other in some environments (for example, soil); their occurrence is also, in general, related to the selective pressure of therapeutic antibiotics use in man or animals. A recent study, observed that a one-week old infant had antimicrobial resistant genes – *tet(M)*, *ermB*, *sul2* and $bla_{(TEM)}$ in its gut bacteria even though no antibiotic treatment had been given. This infant received *Enterococcus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Klebsiella* spp. and *E. coli/Shigella* spp., from its mother during birth and breastfeeding. Some of this transmission is highly likely to have come from the mother's skin, enforcing the point that commensals of the skin are underappreciated, especially with regard to transmission of antibiotic resistance genes (Zhang *et al.*, 2011).

Even though there has been a wealth of information published regarding the Gram-positive resistome on the skin, there is less known about Gram-

negative organisms. Antimicrobial resistance in Gram-negative bacteria from the skin appears to be increasing in prevalence. The mechanisms of resistance involved in these organisms include efflux pumps, inactivation of the antibiotic, mutation in the target protein and outer membrane porin mutations. Once some mobile resistant determinants have been acquired by these bacteria, they become highly resistant with differing mechanisms of resistance being very difficult to thwart (Gootz, 2006).

My research involves characterizing the antimicrobial resistome of Gram-negative bacteria isolated from the skin and nasal cavity of participants from a pan-European multidisciplinary study, which is researching the effect of antibiotics on the emergence of antimicrobial resistant bacteria within the human host. Deciphering the resistome of the microbiota from different sites such as the skin can help elucidate the mechanisms of resistance and the possibilities for these resistances to transfer. Also, more and more antibiotic resistance genes are being discovered in commensals which have the potential to become human pathogens. Because commensals are a reservoir of antibiotic resistance genes, characterizing the skin microbiome is clinically important.

I would like to thank the Society for Applied Microbiology for awarding me the President's Fund Grant for attendance at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 2012 and allowing me to present my research, which was an invaluable experience.

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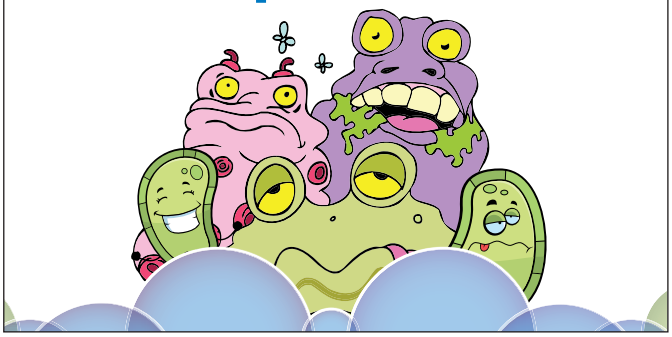
Lisa Bishop
University College London and Animal Health and Veterinary Laboratories Agency



corporate news

The latest news, views and microbiological developments from our Corporate Members

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Cherwell Laboratories offer solutions for a variety of environmental monitoring purposes including the supply and service of the SAS air sampler range. These robust, reliable air samplers are easy to operate and use readily available Contact plates avoiding costly, specialist consumables. Alternative petri dish versions are also available.

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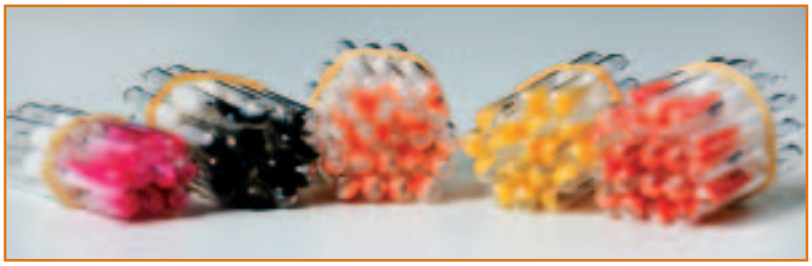

NCIMB strain typing services: MLST

Identification of bacteria to strain level can be valuable when it comes to investigating contaminants in manufacturing environments, comparing them to previous contaminants and tracing their source. It can also be a very important with respect to the industrial use of bacteria.

For example, some companies may patent the use of specific strains within industrial processes and it is therefore vital that the strain can be accurately identified if the patent is contested or if infringement is suspected.

In addition, specific strains of bacterial species are required for the production of probiotic products and this is an area in which we are currently seeing very high demand for strain level identification.

Multilocus sequence typing (MLST) uses the sequences of internal fragments of (typically) seven essential house-keeping genes, i.e. genes required for processes that are essential for cell operation, to characterise isolates, and it has become one of the primary tools that we use.

The data obtained using this technique is in the form of a sequence profile comprising seven allele numbers and strains are identified by comparing the sequence profiles with previously published data. For more information contact Vikki Mitchell.

further Information

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Leatherhead Food Research: food safety & product integrity

Leatherhead's food safety portfolio provides a comprehensive range of products and services to help food and drink companies maintain the highest possible standards of safety and stability in their products. We provide a broad array of microbiological food testing, analysis and consultation covering microbiological food safety, training and advice, and bespoke testing, if required.

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- Microbial inactivation kinetics (to aid food processing)
- Molecular diagnostics including speciation of bacteria, yeasts and moulds, and horsemeat
- Enteric virus research and detection
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- HACCP training and studies including troubleshooting and audits
- Biofilm study and control
- Method development and validation of rapid detection methods
- Validating and advice on cleaning and disinfection procedures

Additionally, our Food Safety & Product Integrity Department is supported by expertise from across Leatherhead. For example, we can check the safety/shelf life of a reformulation, whilst ensuring that it meets the required sensory and nutritional properties. We can also provide regulatory advice so that the product meets the required legislation for the countries in which is it sold.

further information

Visit: www.leatherheadfood.com
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Microbiologics debuts new molecular standards product line — Helix Elite™

Microbiologics, a leading global manufacturer of prepared quality control microorganism products, debuts its first molecular product line, Helix Elite™. These molecular standards are intended to facilitate the development, validation, and monitoring of molecular assays. The Helix Elite™ molecular product line currently includes 13 molecular standards for microorganisms that are difficult to grow or cannot be cultured such as *Cryptosporidium* and *Norovirus*.

These synthetic standards are developed using a unique patented bioinformatic algorithm that combines the genetic diversity of diagnostic sequences from the target organism. Helix Elite™ molecular standards can be used as internal or external positive controls in a defined reaction or spiked into matrices and are applicable for a broad range of assays and instruments.

Microbiologics' CEO Brad Goskowicz commented: "*Microbiologics is leveraging its experience as a global provider of microbial cultures and reagents to provide innovative products of the highest quality to support*

molecular diagnostics. With the addition of Helix Elite™, Microbiologics is now positioned to offer a full-line of controls, from microorganisms and attenuated strains to genomic and synthetic molecular standards."

further information

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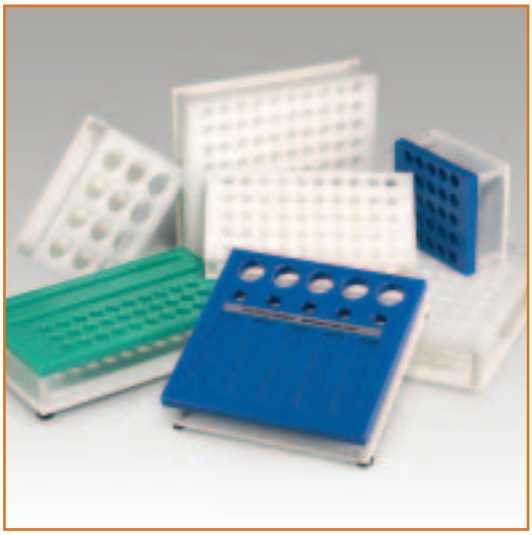

New products from Prolab

Pro-Lab Diagnostics now offer a full range of laboratory racking systems and bench top equipment. Racks can be custom designed to suit all requirements for almost every conceivable container used in laboratories.

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With respect to products required for *C. difficile* testing. Pro-Lab Diagnostics now provides a complete range of products for GDH and *C. difficile* Tox A B testing in lateral flow, Elisa and molecular platforms, samples and demonstrations are available on request.

further information

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Unique twin airlock workstation launched

Now available from Don Whitley Scientific Limited, the Whitley **A55 Anaerobic Workstation** has four gloveports, two airlocks (one at either end of the chamber) and a huge incubation capacity.

This workstation is ideal for two user types:

1. Those who have a high sample throughput and want to improve workflow. This workstation provides the facility to introduce samples through either airlock, process them through the chamber, and remove them from the other airlock at the end of the incubation cycle. Hours can be saved through improved plate organisation.
2. Those situations where two people need to use the workstation simultaneously but need different incubation temperatures. With a special modification, the A55 can be fitted with a partition to allow different temperatures in either side of the chamber, ideal for research applications. This workstation provides a cost-effective alternative to buying two separate workstations.

The A55 can accommodate up to 1,400 x 90mm Petri dishes. A removable front is fitted as standard on the right hand side of the cabinet to facilitate the introduction of bulk samples and equipment that may need to be used inside the chamber. A customised trolley is also provided as standard on this workstation.

further information

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Fecal Transwab® with Cary Blair Medium includes a marked swab that can be used for direct rectal sampling, or for transferring faecal samples into the tube of medium. Selenite Broth is used for enrichment of *Salmonella* from faecal specimens. The swab can be transferred directly from a Fecal Transwab®, or the broth can be used to test any faecal specimen. GBS Broth is supplied with one or two swabs and is used to enrich vaginal and/or rectal specimens for Group B *Streptococcus* during pregnancy.

further information

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information

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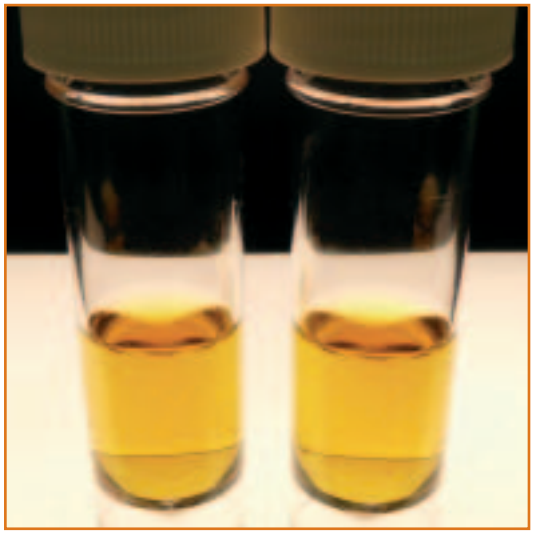
Each Corporate Member of the society may publish ***up to*** 200 words on a topic related to their field of activity in each issue of *Microbiologist*. For further information please contact Nancy Mendoza by email at: **nancy@sfam.org.uk**

Both Corporate Members and Ordinary Members of the Society will find a wealth of useful information and resources in this section.



New selective *Listeria* express enrichment broth also enhances expression of target antigens for downstream testing

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Developed to give improved growth rates of Listeria over traditional selective enrichment media, LEE Broth enhances the expression of target antigens for most commercially available immunological test methods whilst maintaining suppression of non-target organisms.

Key to the efficacy and convenience of Lab M's LEE Broth is the blending of selective components directly into the medium, eliminating any need for supplementation. LEE Broth is formulated to stimulate growth from low numbers of organisms to achieve the high levels required for further testing in just 24-hours.

Lab M's LEE Broth is ideal for enrichment prior to plating on selective media such as Harlequin™ *Listeria* Chromogenic Agar and is highly effective as a secondary selective enrichment broth following primary enrichment. It also performs particularly well as the enrichment step for subsequent rapid testing in ELISA, lateral flow devices or PCR.

Lab M's extensive portfolio of media available for Listeria testing includes: Harlequin™ *Listeria* Chromogenic Agar, as dehydrated culture medium or ready-to-use plates, in addition to a variety of enrichment and selective plating media for use in different protocols.

further information

Visit: www.labm.com
Tel: +44 (0)161 820 3833
Email: info@labm.com

Details for the next BMS Masterclass released

BioConnections are pleased to announce that the next Biomedical Science Masterclass will be held on the 11th April, 2014 at Charles Darwin House in Central London.

The topic for this one day event will be Carbapenem Resistant Organisms with a series of lectures focusing on screening, identification, guidelines and new technologies. These lectures will be presented by Professor Neil Woodford & Dr Katie Hopkins (PHE, ARMRL), Dr Mandy Wootton (PHW, SACU) , Dr David Wareham (Barts Health NHS Trust) and Professor Derek Brown (EUCAST).

Full details including programme and online booking form are available from the BioConnections website at: www.bioconnections.co.uk/BMS-Masterclass.

further information

Visit: www.bioconnections.co.uk
Tel: +44 (0)1782 516010
Email: welcome@bioconnections.co.uk

sfam events in 2014

Save the dates!

2 April 2014

Activated Sludge Meeting

- **Control of waterborne disease: a century of the activated sludge sewage treatment process**

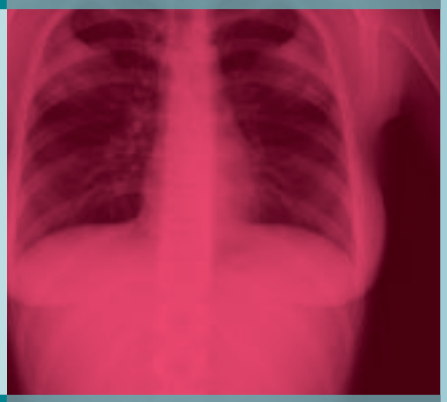
The Lancashire County Cricket Club, Manchester, UK

30 April 2014

Spring Meeting

8th broadening microbiology horizons in biomedical science meeting

- **Control of infection: current status and future prospects**



The Sheffield Hilton Hotel, Sheffield, UK

30 June – 3 July 2014

Summer Conference

- **Zoonoses**
  - In conjunction with the Med-Vet-Net Association
  - **Including the *Journal of Applied Microbiology* (JAM) Lecture**

The Grand, Brighton, UK

For further information on these events please visit www.sfam.org.uk/events
or contact Sally Hawkes ■ Email: sally@sfam.org.uk ■ Telephone +44 (0)1933 382191

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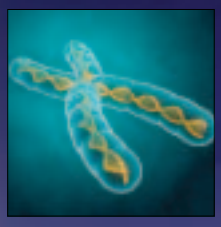

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