# the science of beauty

Vol 9 No 6 June/July 2020

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Refer to our article "Clean, Repair, and Enrich" in this issue.
Learn more about the A S Harrison & Co range of personal care ingredients –
Contact us for more details, starting formulations and samples.

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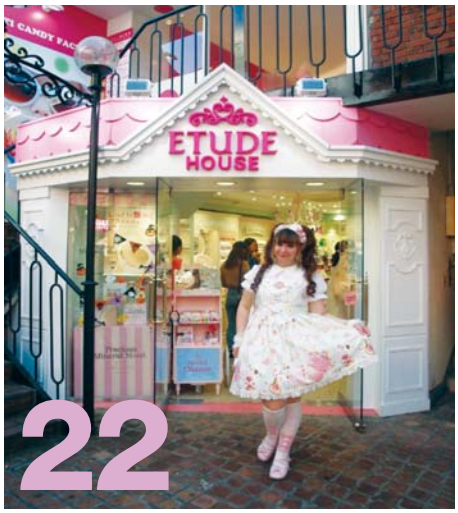
June 2020


## Business



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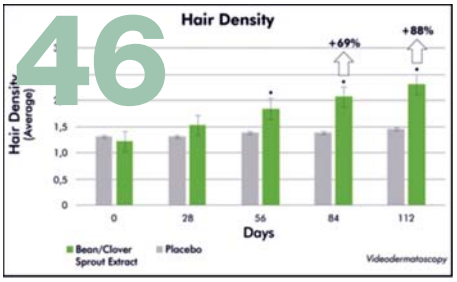


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## THE COUNTDOWN IS BACK ON! 4 MONTHS TO GO

I am sure that everyone will agree that the world as we have always known has changed forever. What we have all had to face over the last few months is something most of us would never have dreamed could ever happen. But as individuals, businesses and industry as a whole we have had to change and adapt to a continually evolving landscape.

If this were any normal year we would have just finished our 2020 Conference and we would now be celebrating our technical award winners, starting to put into practice all the new Sustainable products we learnt about and remembering the good times we spent catching up with old friends. However this is not a normal year…

As part of the conference organising committee and ASCC Council member, I remember back to the discussions that occurred in March at the start of it all as we were coming to terms with the prospect of potentially postponing or cancelling our Annual Conference for what I believe would be the first ever time. As a testament to the society and organising committees of the past 50+ years we have been able to continue to hold our Annual Conference year after year despite all manner of potential roadblocks. The decision to postpone this years conference was an extremely difficult one but one as a Society we had to make. We were unsure what road lay ahead and there is still some sense of uncertainty. But as we sit here now and with restrictions slowly loosening both locally and Internationally, the organising committee are confident that our long awaited 2020 Conference will go ahead as planned in November (albeit with potentially some changes). I suppose one side benefit of holding the event in November rather than May would be potentially a better weather outlook!

As we get closer to the event we will continue to monitor the situation and share any updates with everyone. The good news is that we reacted quickly to secure revised date for the conference as well as ensuring all existing arrangements were transferred across to the new timeline. You will start to see notifications regarding speakers, exhibitors and other key pieces of information flowing through in the next few months.

Registrations will still be open until the middle of October so we would encourage everyone to register if you have not done so. You can find the booking form for the conference at https://events.ozaccom.com.au/ascc-2020/registration.

I would like to pass a big thank you to all our Premium Sponsors, General Sponsors, Exhibition Booth holders and Confirmed presenters. Without your continued support even in these uncertain times we would not have an event. To the Conference Organising team I apprecaite your hard work and dedication even with everything that has come along. Your determination to continue and push ahead with organising the event even with everything changing day to day keeps me inspired to see this through to the end and make it our best conference yet!

**Matthew Martens- ASCC 2020 Conference Chairperson**

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**To keep updated with all the latest conference information make sure you visit www.ascc.com.au**

**52nd Australian Society of Cosmetic Chemists Conference**

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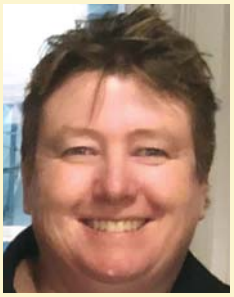
# meet the team...

**WENDY FREE** has degrees in Science (B.Sc) and Technology Management (M.Tech Mngt) and is a member of a number of industry associations including Australian Society of Microbiologists, Royal Australian Chemical Institute, Association of Therapeutic Goods Consultants and is a Fellow of the Australian Organisation for Quality. With more than 25 years industry experience, Wendy's current roles include APVMA GMP auditioning, contributing to the Cochrane Collaboration and on a day to day basis, Scientific Director Quality Matters Safety Matters Pty Ltd (QMSM) that has over the last decade Wendy has provided expertise to over 400 Australian and International businesses. She specialises in regulatory compliance, commercialisation, troubleshooting and GMP systems, and considers cosmetics amongst the most challenging and enjoyable part of her work.

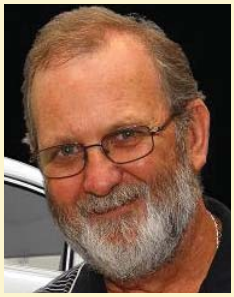
**JULIAN JONES**, the founder and Managing Director of ikonsulting Pty/Ltd, is Passionate about the Personal Care Industry in Australia and Globally.
Julian has been an active member of the ASCC for over thirty years. During this time he has served as President and Chairman of the Victorian Chapter of the ASCC. He is widely known and well respected both nationally and internationally for his knowledge and skills in developing and marketing the best Personal Care Products.

**JOHN STATON** has a background of over 40 years experience in the pharmaceutical and healthcare industries. John is a life member of the ASCC and serves in a number of industry representative roles with ASMI, ACCORD, TGA and Standards. He is the Australian representative to the ISO Committee on Sunscreen Testing-TC 217. (The committee for development of sunscreen standards). John is also in demand as a speaker on the International Conference Circuit.

**MICHELLE KANE** is the managing director of PharmaScope Pty Ltd, a privately owned contract manufacturer established in 2004. Michelle has over 30 years experience in the pharmaceutical and personal care industry, being involved at many levels from procurement, product development, manufacturing, financial management and staff training and development, to name a few... Being based on the West Coast always brings the added challenge of seeking niche product development solutions and working creatively to achieve manufacturing outcomes in a competitive marketplace for our clients global demands.



**PAM JONES** has worked in the Personal, Homecare and Pharmaceutical markets for more than 30 years. She has been working out of Asia since 1996 and is well versed and connected with the Asia Market.
Her experience covers technical, sales, marketing, management and training roles. She has qualifications in Chemistry, Marketing and Management. Her company PCA Consulting is well known for its training programmes. Pam has worked with and consulted to companies such as ICI, Croda, Ashland, Huntsman, Reed Exhibitions (in Cosmetics) and Connell to name a few. She is currently serving on the ASCC Technical Committee and volunteers as Technical Editor for this magazine.



**RIC WILLIAMS** was educated in Sydney obtaining his Bachelor of Science in Pure and Applied Chemistry from the University of New South Wales (1980) and a Diploma of Environmental Studies from Macquarie University in 1983.
Ric has had 40 years experience in the industry working for many companies and operating his own consultancy business for many years.
He has presented many lectures and workshops at national conferences for the Australian Society of Cosmetic Chemists (ASCC), the Association of Professional Aestheticians of Australia (APAA), Cosmetic and Pharmaceutical Special Interest Group (CAPSIG) and also beauty colleges nation wide.

**MARG SMITH** is the owner of Syndet Works – an Australian company established in 1984 to formulate and produce soap free skincare bars. Syndet has developed an enviable reputation for custom formulated and manufactured skincare that now extend well beyond the origins of the business.

**JEN SEMPLE** is Innovation & Education Manager at Accord Australasia, the peak national body for formulated chemical products. She is passionate about communicating the benefits of our industry's products to wider society and has authored a number of public education websites such as furphies.org.au, sunsible.org.au and hygieneforhealth.org.au. Jen also manages Accord's sustainability initiatives and seeks opportunities to build relationships between industry and academia. She has a PhD in Chemistry and Graduate Diploma in Education, and is a member of the Royal Australian Chemical Institute.

**EMANUELA ELIA** is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

**JAMES GILLARD** is the Principal of Insurance Made Easy whose services include – business insurance, travel insurance and financial services. Insurance Made Easy has a client list of over 2000 businesses from all industries. The relevant major insurance schemes are – Hair and Beauty, Pharmaceutical Companies and Natural Therapists.

**STEVE WELSH** is a cosmetic packaging specialist with over 20 years experience across all mediums of packaging. As the director of Weltrade Packaging, Steve leads a team of designers, technicians, printers and supply chain professionals. To ensure the best exposure of your beauty, skincare or cosmetics brand. Steve's philosophy is to design your packaging correctly, right from the start, so you can elevate your brand and move more product. Steve works closely with leaders in the cosmetic industry to ensure that your packaging consistently stands out on the shelves within this highly competitive market.

**TINA ASPRES** has worked as a Pharmacist for almost 20 years in retail, industry and academia as well as being a Cosmetic Chemist. Currently she works in industry and has vast experience in both the pharmaceutical and healthcare arenas. In addition to this she is a casual academic at UTS, School of Health, (Faculty of Pharmacy in Pharmaceutics). Tina has a great interest in clinical research in dermatology and the treatment of skin disease and conditions and is Clinical Trial Coordinator at South West Sydney Dermatology. She is a keen researcher in transdermal drug delivery systems. Tina is a Member of the Pharmaceutical Society of Australia and a Member of the Australian Society of Cosmetic Chemists. She regularly consults pharmaceutical companies in the area of acne, eczema and skincare especially in the area of cosmeceuticals and has devised and written numerous support, training and education material for companies aimed at both professionals and consumers. Tina consults for the Eczema Association Australasia and is on their Integrity Assessment Panel and has worked with Choice Magazine on numerous reports. Tina has presented at the Annual Scientific Meeting of the Australasian College of Dermatologists and has published within the pharmacy and medical literature in the area of sun protection, Vitamin D, skin cancer prevention and eczema as well as co-authoring the book 'All About Kids' Skin – The Essential Guide' published by ABC Books

**GINT SILINS** is a registered patent and trade marks attorney, and a principal of Spruson & Ferguson Patent & Trade Mark Attorneys (incorporating Cullens). He holds a Bachelor of Science degree in chemistry with honours in biochemistry, and a Doctor of Philosophy degree in biochemistry. Gint specialises in protecting branding and innovations largely in the health care, personal care, animal health, food and beverage, biotechnology, industrial chemical, clean energy and agricultural sectors. His practice includes: conducting brand and innovation availability and registrability searches; IP audits; registering patents, trade marks and designs worldwide; enforcing intellectual property rights; resolving IP disputes; and, providing infringement and validity advice.

# when your customer **isn't** happy… turn **this** ☹ … into **this** ☺

**by Julian Jones**

As suppliers of goods and services we all work hard to win new customers. It's a fact that it takes a lot more work, time and money to get new customers than it does to keep existing ones! We all love selling to happy clients and we do everything we can to keep them happy.

Inevitably though, there will be times when for a myriad of reasons, one of our clients is unhappy with our offering. It may be the perceived quality of the product, the timeliness of the products arrival with the client, suitability issues or any number of other reasons. We are told that the customer is always right and although there are clearly times when they aren't, as business owners, we need to exercise some flexibility when confronted with a customer complaint or issue.

One way to think about a customer who complains is to think about the effort required to lodge that complaint with us. I have heard it said before that if an unhappy customer bothers to contact your business to raise their concerns, it's a great thing, as they care enough about your product or service to tell you about it! They haven't given up on you and walked away, never to return and possibly about to tell any number of people about their negative opinion of your product or service! In reaching out to your business, they are actually saying, 'I have a problem and I'm inviting you to work with me to solve it in a mutually acceptable way'.

Now I know sometimes the language used to make you aware of their concern may be less than respectful but regardless of that, they are giving you the opportunity to engage with them to understand the reason for their complaint and to negotiate a win/win outcome for both of you. Rarely is a customer complaint unresolvable even in these times of anonymous social media communication. I will say, though, that the first piece of information you as a business owner needs is a confirmed set of contact details!

Prompt response systems built into your customer service department are critical to resolving most complaints quickly and efficiently. When a customer bothers to advise you of an issue, they are expecting a fast, professional response, ideally from a member of your team who is empowered to resolve the issue themselves.

That person should have a well-developed sense of empathy and be able to quickly understand and identify the issue, determine the best way to resolve it, and then take action to do so. Return policies, refund policies, repair policies are all great on paper when you are defining the potential risks and costs associated with your products or services, but a level of flexibility, coupled with allowing your customer service contact people a level of autonomy, can quickly turn unhappy clients into satisfied, unpaid sales reps for your business!

In my experience, when a company gives me great customer service when I'm not happy with them and meets or exceeds my expectations, I can't help but tell all my friends and associates what a great business they are!

To use a well-worn cliché, in closing … turn that frown upside down!

Till next time…

*Cheers,*

**Julian**

# a time for greatness for Australian manufacturing… but will we make the most of it?

by Michelle Kane

I have a wide ranging group of very opinionated friends, from many different ethnic backgrounds, diverse skillsets, contrasting educational and social environments, varied work places but the one common theme when discussing Covid 19 has been the collective we must support Australian manufacturing call to arms.

The question is, will you and I and the country actually translate this call into real and sustainable actions into the future to facilitate the growth of Australian manufacturing ? And importantly, how much are you prepared to pay for it? I know my answer.

There is no doubt that Australian manufacturers of all industry types raised their hands to be involved in tackling Covid19. Over 2,100 submissions were received by one industry body alone from organisations willing to support government in response to the current pandemic. This included manufacturers from all states and territories. Within our industry the redirection of manufacturing resources into the production of hand sanitisers was obviously a stand out. But we need vision, and common sense to see all of this equate to future growth in manufacturing in new areas and the bringing back of some industries, or at least skill sets already lost.

Manufacturing in Australia directly and indirectly employs over 10% of the population. The manufacturing industry outputs amount to over $110 billion annually, or equivalent to ~6% of Australia's Gross Domestic Product.

My group of friend commentators and experts alike agree that the world will be a different place after the COVID-19 pandemic passes and this statement rings true for the Australian manufacturing industry including pharmaceutical, cosmetics, packaging and raw materials.

We have witnessed how rapidly the manufacturing industry has mobilised to help fulfil critical needs and are quickly realising how vital it is to protect and develop our sovereign manufacturing capabilities.

Business needs to be resilient – that is my firm belief. Building a resilient manufacturing business is key to managing volatility, in whatever face it reveals itself – even a global pandemic.

Did you know that the average Australian manufacturing industry swells 20% above its trend size in upcycles and 20% below size in downturns – The loss of just one customer would have moderate to significant impact for 30% of businesses and for 10% would force them to close down according to one recent study. Just losing one customer and 10% of manufacturers would close!

So to support the call to arms I believe we all have a key part to play – consumers, manufacturers and government.

Consumers must support local manufacturing. They must educate themselves about where things come from, ingredients, packaging, accept

that the cost of labour is high, accept that we are a big country that needs everything freighted, accept that to make it here may well cost more. They must embrace clean and green as cheaper energy alternatives as that will help. Consumers must spend their dollars with companies that manufacture in Australia after they have educated themselves about what local (Australia) actually looks like.

Manufacturers must make themselves resilient. We must look internally and investigate what drives resilience, which when combined with competitiveness will enable long term performance, up skill our workforce and celebrate innovation. We must be ambitious and lobby for focused manufacturing policy interventions. Every manufacturing company has the opportunity to succeed, even in the toughest of times, if it adopts a resilience strategy.

The federal, state and territory governments need to ensure access to supply chains are guaranteed to the manufacturing industry. They must deliver a nationally consistent approach and coordinated response and provide greater support and resources for business continuity.

As a manufacturer I implore every Australian to take a bold leap of faith in this industry and know that Australia's manufacturing success can be based on innovation, quality, and productivity — not just trying fruitlessly to catch low-wage exporters in a race to the bottom in labour costs and standards.

Whilst I may be personally scarred from this experience and NEVER want to see another hand sanitiser formulation again, this country needs to make things. This crisis has demonstrated exactly that. Let's get on with the job of making cosmetics and pharmaceuticals, personal care products and well to be honest gin…time for all you distilleries to go back to that…I still have my tonic water left from the last article. ☺

MICHELLE KANE is the managing director of PharmaScope Pty Ltd, a privately owned contract manufacturer established in 2004. Michelle has over 30 years experience in the pharmaceutical and personal care industry, being involved at many levels from procurement, product development, manufacturing, financial management and staff training and development, to name a few... Being based on the West Coast always brings the added challenge of seeking niche product development solutions and working creatively to achieve manufacturing outcomes in a competitive marketplace for our clients global demands.

# Making sure you know the **difference** between

## Product Liability & Public Liability insurance and (What is covered with Product Recall Insurance.)

by James Gillard

Just like Apples, Oranges and Bananas these three insurance covers are quite different under a General Liability insurance policy that you may already have or are thinking of purchasing.

This article sets out to explain each insurance cover so you are clearer in your understanding and the type of events which may trigger an insurance claim under each.

### Product Liability Insurance – what is it?

Your Product (itself causes harm) means any physical property after it has left the Insured's custody or control, which has been designed, specified, formulated, manufactured, constructed, installed, sold, supplied, distributed, treated, serviced, altered or repaired by the Insured or on the Insured's behalf .

### So, what event would trigger a likely insurance claim?

The Product would need to cause or alleged to have caused Personal Injury or Property Damage.

Clients therefore purchase this type of insurance as a way of reducing their liability in the event their product causes harm. The outcomes of such events can be costly to any Business. If such an occurrence takes place and there are multiple units of the product already sold that could potentially cause harm, it may be necessary for the insured to initiate a recall, so potential further injury or damage to property cannot occur.

The majority of Public and

Products Liability insurances do not automatically include Product Recall and it may be worthwhile considering a Stand-Alone Product Recall Insurance program.

## What are the type of Product Recall expenses that can be covered under a Stand-Alone Product Recall Policy?

- The costs associated with recalling the product from the market
- The cost to replace the product in the market
- Any extortion costs made against the product
- Expenses about rehabilitating your Business due to the Product Recall
- The costs associated with the employment of specialised consultants such as Public Relations experts
- Business Interruption cover to protect your loss of profits during the recall which has impacted the Businesses productivity
- Some insurers also provide In-house claims personnel available 24-hours-a-day,
- 7-days-a-week, dedicated to crisis management claims

## So, what is Public Liability Insurance?

Public Liability Insurance protects your business for any negligence which results in either physical injury or property damage. With society becoming more litigious it is important that you properly protect your business activities whether they are conducted on or off site.

A claim could result from one of your clients tripping over your equipment whilst at their work premises, suffering a loss of income due to time off work or significant permanent injury.

A physical personal injury could mean bodily injury, sickness, disease, disability, shock, loss of amenities, discomfort, disfigurement, malformation, fright, mental anguish, mental injury, or death of or to any person. It might be the effects of false arrest, false imprisonment, wrongful detention or malicious prosecution or the effects of wrongful entry, wrongful eviction. It may also be how the aggrieved party has been impacted by libel, slander, humiliation or violation of personal rights and the effects of assault and battery committed for the purpose of protecting persons and/or property.

As for Property Damage this could mean loss or destruction of, or physical damage to tangible property, including any resulting loss of use of that property.

## One call to Insurance Made Easy -Insurance Brokers

If you would like to learn more about General Liability Insurance (including Product Liability & Public Liability Insurance), and Product Recall Insurance you can contact our friendly team at IME Insurance Brokers.

We provide professional service and personal assistance when we discuss what your individual circumstances are.

You can contact us by calling our office on 1800 641 260

**James Gillard**

*Managing Director*

# impact of the pandemic on *in-vivo* cosmetic studies

by Emanuela Elia

In 2020, the world is facing a challenge that many of us would have never predicted. All the best wishes exchanged a few months back for a new year filled with prosperity and good health, have suddenly been swept away by a terrible pandemic. Many lives have already been lost, many more will, and still more will have their lives changed forever. Running studies, however, is still essential to the cosmetics industry. The focus now is determining how cosmetic studies can be adapted in the safest possible way, while maintaining the scientific integrity of studies with similar timelines and budget constraints.

## Restrictions

Authorities in the different countries struck by COVID-19 infections have taken different measures in response to the pandemic, ranging from long lockdown periods to no shutdown at all (e.g. Sweden). Restrictions on travel, gatherings, opening of businesses and services, social distancing, use of personal protective equipment, and increased attention to sanitation and hand hygiene have been the most frequent and effective measures. Naturally, the risk of getting infected, together with the measures imposed by the relevant authorities, have either prevented or discouraged to some degree study volunteers from taking part in studies that require visits to the study clinic. These restrictions have also dissuaded research clinics from conducting study visits.

## Ethical reasons

COVID-19 is a potentially deadly virus which can spread very easily if appropriate precautions are not taken. Evidence has shown it to be particularly dangerous for the elderly and people suffering with other health conditions. At the time of writing this article, a vaccine or cure for this virus is not yet available. As a result, protecting the members of our community is everyone's responsibility.

While certain activities considered essential should continue to be carried out with appropriate precautions, some ethical considerations come into place with regards to taking part in studies investigating non-health related outcomes. This is the case of cosmetic studies, where the objective is to beautify skin, hair, and nails, rather than prevent or cure. For this reason, with or without specific restrictions, many companies across the globe have refrained from conducting cosmetic studies that could unnecessarily expose the community to the risks of spreading the virus. These companies have been dealing with the frustration of being unable to work with their usual research service providers to collect in-vivo efficacy and safety evidence for their skincare products.

## Adapt your studies

Where possible, we have seen businesses of all sorts, from retailers to service providers, responding to the pandemic by working remotely

where possible. These activities include meetings, signing of documents, purchases, and health appointments. We have realised we already had the tools to do all of this before it suddenly became a necessity.

The same applies to clinical research. Consumer studies and virtual clinical trials have been popular for quite some time. These allow volunteers to take part from the comfort of their homes and report outcomes to be assessed remotely. The aim of these studies is to collect evidence based on subjective assessment of the volunteers who took part in the study or of the expert assessor (e.g. doctor, trained professional) where it is possible to do this remotely. There are some limitations to these types of studies, mainly related to the lack of objective assessments which normally require specialised equipment or other validated procedures that can only be employed in clinic. Nonetheless, these studies are especially important from a marketing perspective and are gaining momentum during the current emergency as a valuable alternative to in-person trials.

There are some trials that cannot be modified to become home based studies as they rely on particular equipment to be used during face to face appointments. Ongoing trials such as these may suffer significant delays due to lockdowns and other aforementioned restrictions. In extreme cases, these studies might be cancelled due to the impossibility to collect relevant data – in this case, repeating them at a later date might have to be considered. For the vast majority of trials, however, there are other options that can be considered.

## How do virtual studies work?

In contactless studies, products are sent directly to volunteers and questionnaires are completed online. Face to face visits are not required. Apart from these key changes, studies mirror those run in-person.

Typically, these types of studies are used to support claims such as "X% of users thought their skin elasticity improved", "X% of users saw an improvement with the appearance of fine lines and wrinkles", "X out of Y would buy it again", and so on. Cosmetic companies that can base their claims on the subjective evidence rather than objective evidence, could still conduct studies during the pandemic.

Secure electronic documents can be set up quite easily and used throughout the different stages of the study, from obtaining volunteers' consent to data collection. Another great advantage of electronic data capture is that it eliminates the need for data entry, saving time, reducing data entry errors, and eliminating the need for double data entry. Collected in this way, data is soon ready for statistical analysis. This can speed up project timelines substantially.


## Patience is key

Having to alter schedules and make changes to R&D timelines due to the pandemic is certainly frustrating for cosmetic companies. The same frustration is experienced by research clinics who might not be able to complete or carry out certain studies they have worked hard to get started. However, the current situation is quite extraordinary and everyone is required to apply their best judgment in all circumstances involving social interaction. When conducting in-vivo cosmetic studies, we should allow some level of flexibility to adjust to the 'new normal'. We have to act sensibly to minimise the risks we are facing at each given time, because we are equally responsible for our actions and their consequences. After all…we are in this together!

**EMANUELA ELIA** is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

# platelet rich plasma

## the real deal

by Tina Aspres

The worldwide market for PRP grew from around $45 million in 2009 to $120 million in 2016 and is forecast to exceed a staggering $4.5 billion by 2024. It has been promoted as capable of growing hair, smoothening acne scars, reducing the appearance of striae, eliminating fine wrinkles and slowing down the ageing process. With an excellent safety profile across all skin types, the mention of PRP immediately generates an unquestioned feeling of a natural effective treatment that requires no further explanation. Too good to be true? Just clever marketing? Or backed by science? This article briefly outlines PRP, its mechanism of action and the clinical evidence supporting its purported benefits.

PRP by definition refers to a concentrated autologous preparation separated from the patient's own blood and then applied either topically or injected intradermally to achieve a designated benefit. Most authors agree that "therapeutic" PRP requires a minimum concentration of one million platelets in one millionth of a litre (microlitre) which is approximately five times that of normal whole blood. Commercially available systems claim concentrations 2-8 levels higher than baseline but unfortunately no quality head to head comparison studies exist to determine the ideal concentration required for maximal clinical benefit.

Understanding the mechanism of action of PRP requires focusing on the tiny blood cells (platelets) that help our body form clots to stop bleeding. Within a few minutes of aggregating around a clot, platelets secrete hundreds of substances from within their cytoplasmic granules, peaking in 30-60 minutes before tapering within one week. Platelet granule types are alpha-granules (commonest), dense granules and lysosomes. The dense granules primary role is haemostasis and coagulation whilst lysosome granules function to digest bacterial proteins. The crucial granule in "regenerative medicine" is the alpha granule which contains an abundance of signalling molecules (growth factors, cytokines, chemokines) that trigger downstream intracellular signalling cascades that alter gene expression and protein synthesis.

Another source of complexity is the fact that PRP is not just a single "product" instead containing more than just platelets suspended in plasma. Depending on the techniques and/or device used for separation there are varying amounts of white blood cells, red blood cells and fibrin (a major component of the blood clot). Of these additional cell types white blood cells may be equally and potentially more important than platelets above a certain as yet unknown threshold. White blood cells provide not only antimicrobial effects but high concentrations of matrix metalloproteinases that play a key role in angiogenesis, extracellular matrix remodelling and anagen hair cycling. PRP may be classified as pure PRP, leucocyte (white blood cell) rich PRP, or leucocyte poor PRP but many studies fail to outline PRP

# or a clever con?

components, concentrations and volumes so interpreting and reproducing results is difficult.

The most abundant research supporting PRP in dermatology exists in treating alopecia. PRP induces proliferation of dermal hair papillae cells, stem cell differentiation, neoangiogenesis around the hair bulb and decreases perifollicular microinflammation. In androgenetic alopecia (male and female pattern baldness) most studies support a positive benefit although the minority present data using a quantifiable measurement. The following observations can be made after reviewing the current literature: ongoing treatment is required to prevent regression of the condition, injection intervals is important with a regime of three monthly injections and then 3-monthly maintenance showing the greatest statistical significance and clinical best responses, injection depth appears crucial with subcutaneous (subdermal) injection of 0.2-0.5ml volumes per 2-3 cm most beneficial due to greater diffusion through the looser subcutis allowing more widely spaced injection points, better diffusion, reduced pain and associated better compliance. The ideal candidate for PRP treatment ideally has onset of androgenetic alopecia after 25 years of age as this correlates with less aggressive alopecia than teenage onset disease, and disease duration less than 2-5 years. Smaller numbers of studies suggest potential improvement in other disorders if hair loss such as alopecia areata and scarring alopecias but there is insufficient quality data to verify ideal preparation, dosing or injection technique in these conditions.

The second most literature supported use of PRP is in post-procedure wound healing and scar management – in particular acne scarring. In investigating post procedure wound healing split design studies comparing fractionated laser ablation combined with topical PRP versus laser alone have shown faster wound healing, shorter duration of erythema, a thicker epidermis and denser more orderly dermal collagen bundles. The addition of PRP by reducing the risk of side effects allows more aggressive treatment settings and associated better clinical outcomes.

Intradermal PRP monotherapy for acne scarring has been shown to be effective as monotherapy and split face studies comparing 1.0-1.5mm microneedling plus topical/intradermal PRP to microneedling alone in acne scarring, report consistently superior results. Combination treatments including chemical reduction of scar tissue, subcision, intradermal PRP and microneedling or fractionate laser produce the highest clinical success statistical significance.

Evidence is increasing that PRP may improve the clinical appearance of striae distensae (stretch marks). Serial monthly PRP injection provided greater clinical benefit than 0.05% topical daily tretinoin or microdermabrasion. Combined intradermal PRP followed by microneedling over topical PRP may prove to be the best therapeutic combination based on more recent studies.

With its inherent high concentration of growth factors crucial in extracellular matrix homeostasis, PRP has aroused great interest as an antiageing tool. Despite reports of improvement of periorbital rhytides and histological evidence of increased collagen density, the current evidence remains less than that for hair restoration, scar revision and post treatment wound healing. The ideal candidate to achieve maximal histologic and clinical improvement is one with photo-ageing aged less than thirty years, with efficacy decreasing with age between 30-40, with older than 40 showing the least clinical response.

There is great interest and potential in the use of PRP in cosmetic medicine. The number of publications on the benefits of PRP and its' presence in mainstream dermatology conferences and textbooks is continuing to increase rapidly. Despite its increasing popularity, there are a number of fundamental gaps in the existing literature such as the lack of a known ideal concentration range, the broad variability in PRP preparation techniques, ideal dose regimes and injection depths for each indication and the lack of large scale double blinded, controlled trials. Ten years ago, PRP was treated with scepticism but as the research continues to build it may prove to actually be the real deal.

*'A global marketplace – local and international opportunities and responses'*

# Off-the-charts hand sanitiser demand brings challenge and opportunity

**by Jennifer Semple**

Hand sanitiser has been a hot, hot topic in recent weeks. Everyone wants it. Market shortages are affecting diverse users: you may have been affected personally by the empty shelves, or professionally as you have struggled to source hand sanitiser for use in your workplace or business.

COVID-19 has helped propel our industry – the traditional supplier of hand sanitiser – into the spotlight as a trusted supplier of essential goods.

Long-term suppliers of hand sanitiser are busier than ever. And some companies are pivoting to hand sanitiser supply and grappling with supply chain, WHS and other essential safety requirements relating to manufacturing and shipping highly flammable goods.

A lot has been happening behind the scenes in the local hand sanitiser space.

The Hand Sanitiser Industry Roundtable was established by federal Industry Minister the Hon Karen Andrews MP to address potential roadblocks for hand sanitiser supply. Accord, an active Roundtable member, conducted a broad industry survey identifying the main supply roadblocks for local manufacturers. For example, 75% and 58% of responding companies reported supply shortages for ethanol and gelling agents, respectively, and supply issues relating to packaging materials were also revealed.

Connect was established following the survey, matching urgent requests for alcohol-based hand sanitiser product with product suppliers. It is an efficient solution developed by Accord in collaboration with the Department of Industry, Science, Energy and Resources (DISER), the Australian Distillers Association and the Consumer Healthcare Products Australia.

Accord has also been engaging on product safety, efficacy and compliance. For example, our discussions with the ACCC and TGA are highlighting potential alcohol-based hand sanitiser efficacy issues and confusing product presentation, and our information sheet 'Safe Manufacture, Transport and Storage of Alcohol-based Hand Sanitisers' provides a helpful resource on key regulatory obligations for companies new to hand sanitiser manufacturing.

Accord is also providing advice to the public on effective and safe product use. For example, following a spike in child exposure incidents involving hand sanitisers, we have included specific messaging on our Hygiene for Health and Children and Household Product Safety webpages on how to keep children safe. And, we are requesting modification of the Department of Health's public health messaging in relation to non-alcohol based hand sanitisers.

As restrictions are progressively lifted, our industry will remain as important as ever to maintaining levels of hygiene essential to combatting any future spikes of infection. Accord will continue to work actively with all the relevant regulators to help ensure appropriate quality and messaging relating to hand sanitisers and other essential cleaning and hygiene products.

Accord Australasia is the peak body representing companies operating in the cosmetic, fragrance, personal care and toiletries sector – from multinationals to small Australian-owned businesses, importers to local manufacturers. www.accord.asn.au

# CLEAN, REPAIR, and ENRICH

We know how harsh some of the handwashes and sanitisers that are currently out in the market. Everyone is, rightfully, washing and sanitising their hands these days. We also know the damage a lot of washing and sanitising can cause to the skin barrier. However, these two factors are seemingly at odds, because a broken skin barrier is more susceptible to infection.

We believe there is a 3-step process to combating these harsh effects – CLEAN, REPAIR, and ENRICH. We have listed below some of our favourite products that will help you formulate gentle, yet effective products to not only take care of our hands, but also our faces.

## CLEAN

It is important to make our cleaning products as mild as possible. Hydresia® SF2 is a truly natural emulsifier providing powerful skin hydration and an active-delivery system, that can also be used as a drop-in deep moisturising additive for hand wash and hand sanitiser formulations. Needless to say, it ticks the palm-free, vegan, non-GMO, PEG-free, COSMOS certified boxes also. It is about as "free from" as you can get, while still conferring real performance and a gorgeous skin feel.

A great emulsifier can add luxury to the first touch of moisturiser to our skin, and unique emollients can continue that journey for our consumers. Likewise, dense, rich foam in a sulphate-free wash product will make them feel just as special and avoid the harsh results on the skin.

AQUASILOIL® Sweet Almond Oil acts as a foaming, cleansing, soothing and healing active. It is a natural oil which is perfectly solubilised in aqueous phase without any use of solubiliser or emulsifier. This PEG-free product offers various benefits like moisturisation, soothing, repairing, protection, collagen synthesis that are derived from base (the original oil used and the lysine). AQUASILOIL® Sweet Almond Oil is used in shampoos, shower gels and cleansing emulsions.

LexFeel™ 7 is a dry and velvety ester with ultra-dry initial feel and subtle after-feel. LexFeel™ 7 is a safe alternative to cyclomethicone. LexFeel™ 7 has excellent spreadability and reduces tackiness in formulas with heavier emollients and, in hand sanitisers!

## REPAIR

Once we have clean hands, it is

most important to repair. Olive leaf extract has been incorporated in the diet as an extract, tea, and powder (in consumable products and cosmetic preparations). The leaf contains a wide variety of bioactive compounds that are beneficial for health and wellness. The olive leaf is full of natural antioxidants, the most prominent being Oleuropein and Hydroxytyresol. When applied topically, it can potentially see a reduction of skin erythema (redness) and an improvement in skin blood flow and dehydration.

What makes oleFreshTM unique? oleFresh™ is a superior quality olive leaf extract formulation made with fresh olive leaves 100% sourced from Boundary Bend's olive groves in north-west Victoria, Australia.

Produced using a proprietary process, olefresh™ has a unique profile containing over 10 different biophenols, of which oleuropein and hydroxytyrosol are the most abundant. olefresh™ is created using fresh leaves that are processed within 4 hours of being picked.

Vegelatum® vegetable gels are proven, functional alternatives to petrolatum in a variety of personal care formulations. They consist of a proprietary vegetable gel technology using natural ingredients. They can also be used to build viscosity and improve feel of anhydrous formulations. It helps to keep hands moist, for a longer time.

COSMELENE®, an active ingredient in concentration similar to that of the fresh Centella Asiatica plant, improves skin's moisture by increasing collagens I and III synthesis. The skin is smoothed, the number and the depth of fine lines and wrinkles is reduced.

## ENRICH

However, it is not just our hands that are feeling the ill effects of these changing times. We are all spending more time in front of computer screens and maybe not getting the amount of sleep we desire.

SOLIBERINE® is rich in phenylpropanoids, and ultra-concentred in verbascoside and echinacoside and protects the skin against the damaging effects of light rays, UV rays, infrared rays, and blue light, preserving the skin's youth, quality, and radiance.

CERNILYS® helps to soften dark eye circles by acting on the genes that control the vascular system. It decongests, calms, reduces hypervascularisation and pigmentation resulting in the eyes looking is fresher, more rested and younger.

*Feel free to get in contact with the team at A S Harrison & Co for a hand with samples and other suggestions at email performanceingredients.ash@harrison.com.au or call us on +61 (0)2 8978 1016*

# Color that Treats Hair Kindly


Consumers are increasingly mindful about what they apply to their hair, wanting cleaner, natural ingredients that provide great results without damage to their scalp and hair strands. Those who dye their hair with permanent hair color also want perfect color that lasts, effectively covers grays and is easy to use for at-home hair coloring.

Lubrizol Life Science – Beauty (LLS Beauty) has launched its first sustainable hair color solution, Naturange, a selection of hybrid permanent hair color formulations, crafted with up to 88% naturally derived ingredients for gentle, long-lasting results.

Naturange offers the ideal solution with its combination of botanical extracts and naturally derived ingredients using Lubrizol sustainable proprietary technologies that provide vivid color and gray coverage, while preventing damage to hair.

Lasting up to 20 washes, Naturange formulations are free of ammonia, parabens, PPD and not formulated with resorcinol. All have a non-drip, creamy texture that is quickly absorbed into the hair for maximum moisturizing and conditioning benefit. No residue is left behind, and hair is easily detangled while it is still wet.

With Naturange, consumers have a hybrid permanent hair color plus treatment formula that creates long-lasting color, even on grays. It also soothes hair and makes it smooth and soft.

Naturange falls at 88% on the Renewable Carbon Index and comes in four ready-made shades:

- Green Tea Black 2.0 Shade
- Hawaiian White Ginger Brown 4.0 Shade
- Hibiscus Flower Red 6.6 Shade
- Chamomile Blonde 10.0 Shade

For more information, please contact Robert McPherson, Account Manager for Australia and New Zealand, at Robert.McPherson@Lubrizol.com or Tel: +61 (02) 9741 5237.

# a look at South Korea

by Pam Jones

President of the considerably large Cosmetic Scientists of Korea is Dr Wan Goo Cho email: SCSK1968@naver.com. During 2019 the society published several journals in English, introduced a researchers award system and held Spring and Autumn conferences as well as a joint symposium with the Korean Society of Industrial Chemistry. This year due to the COVID 19 outbreak, the society plans to hold their 45th Annual General Meeting of the Korean Cosmetic symposium and the Spring Academic Conference on the 25th June 2020. The conference will be held in Webinar format, reflecting the Corona 19 pest control policy, and is limited to conference board members and verbal speakers for face-to-face attendees. They intend to have some suppliers present for displays; however, these suppliers will be limited in number and also space size to desktop displays only. The aim is to publish spring and autumn conferences, symposiums, and journals in Korea and English for all members.

## The Market in South Korea

We see a very different market in South Korea to one that we are familiar with in Australia. The product range from the two dominant local manufacturers is vast and exceptionally well-formulated and tested. The market growth to 2024 is expected to be app 5%. Companies intend to expand overseas to increase their market share in western countries; we even find the Korean influence in Australia by the top two cosmetic companies.

L.G. Household and Healthcare is the second largest manufacturer in South Korea. They market mid to low end cosmetics via their "FaceShop" brand when they purchased a 90% share in the brand in 2009 to boost their sales. The "Face Shop" has a global presence of more than 2,300 stores in 29 countries. One of their best-known products is Jeju Aloe a soothing and moisturizing aloe vera gel, also available as a mask. Used and recommended by many.


Jeju Aloe SoothingGel

Cosmetic companies in South Korea use actors, actresses and boy and girl-bands for endorsing their products in Asia. L.G. products are promoted via Korean celebrities such as Kim Soo Hyun, a well-known actor.

The company is also backward integrated and makes many active


YVOIRE® Products

ingredients in its Chemicals Division for use in their products. An example is Hyaluronic Acid used in their top-end range of injectables. Dermatologists use the brand Yvoire in France, Italy and Russia.

Amorepacific Corporation is number one in the market. This beauty and cosmetics conglomerate has notable brands such as Innisfree, Etude House, and Laneige, to name a few. These brands are based on stories for the consumer, such as:

Innisfree, the brand name derives from [Irish] poet W. B. Yeats' poem, 'The Lake Isle of Innisfree'. The story behind this brand is that it shares the benefits of nature from the pristine island of Jeju and pursues an eco-friendly green life to preserve the balance of nature. Jeju province encompasses the South Korean island of Jeju in the Korea Straits. It's known for its beach resorts and volcanic landscape of craters and cavelike lava tubes. Products marketed include variants such as Green Tea, Volcanic Cluster, Orchid, Bija, Cherry Blossom, Hallabong (a type of orange), Pomegranate, Seaweed and Lava Seawater, just to name a few.


Laniege brand name comes from the French "La Neige", which translates to "the snow". (a reference to white skin) The brand's flagship products include its Water Bank skincare line, Water Sleeping Mask, BB Cushion foundation and six colour layering lipsticks. The Water sleeping mask


Jeju Lava Seawater Mist



Ampoule delivery system

is targeted for skin with insomnia. It comes with a full range of suggestions on sleeping well, including exposure to the sunlight for 30 min each morning, reducing time watching television and social media such as texting at night. The six colour layering lipstick is for those clients who find it hard to apply the six different colours used for volume gradation.

Etude brand markets to the very young pre-teen and the teen market for girls who want to feel like a princess. The design of Etude House is a romantic and princess-style interior with a pink theme. Employees at the store are dressed in pink princess helper costumes, designed to make the customer feel like princesses. Etude brand also has the obligatory ampoule packaging for skin that needs that extra bit of care, although I am not that sure if pre-teen and teen skin needs all this care. It's a campaign to train the youngest of consumers to take care of their skin with cosmetics at a very early age. Teaching young women to take care of their skin by using cosmetics is by far a safer way than plastic surgery. Plastic surgery is used extensively within urban South Korean culture. Some high school students receive cosmetic surgery as a graduation present, and 30% of women in their 20s have had plastic surgery of some for. South Korea has a very different and extensive market range to what we are used to seeing in Australia.


Etude House in Japan


# Interested in formulating your own range of personal care products?

The 107088NAT Diploma of Personal Care Formulation is offered throughout Australia by Australasian Academy of Cosmetic Dermal Science (AACDS). AACDS is Australia's premier college established to meet all learners educational needs in personal care formulation, dermal therapies and cosmetic nursing.

The Diploma is the ideal qualification for anyone wishing to begin a career in formulating their own personal care products or for those already are in the formulating game who want to formalise their knowledge and skills through gaining a nationally recognised qualification. Other career options include Research and Development Scientists, Production / Compounding Managers and Assistants and Regulatory affairs Personnel.

The Diploma provides a comprehensive approach to teaching the formulation of safe, stable and efficacious personal care products for hair, hands, body and face. It also covers the development of products from the concept stage through to reverse engineering. Learners are taught how to prepare a variety of personal care products, adapt formulations to suit organisational requirements and develop products based on the latest industry advances.

AACDS recognises the need to offer flexible course options and study modes therefore the theory component of this course is offered either fulltime or part time via online study. The theory is supported with the provision of practical activity kits so learners can immediately begin learning to formulate from their own homes. AACDS students are further supported with a hands on two day practical workshops conducted by industry experts in Sydney, Perth and Melbourne throughout the year.

The formulation of personal care products can be as diverse as the companies who offer them and only restricted by the developers imagination. If you are looking for an exciting career in a dynamic industry then studying the Diploma of Personal Care Formulation is for you.

Loans and other payment options are available to study this course. Apply today by contacting enquiries@aacds.edu.au or visit our website www.aacd.edu.au

**Linda Sim** **MBA, Dip Cos Science**
CEO of Niche Education Group T/A AACDS

# the cosmetic packaging industry post **COVID-19**

**by Steve Welsh**

2020 has been a very different experience for all of us and it has been a very different year to what everyone had originally envisioned. If you read our previous article in SoB, the COVID-19 pandemic restrictions were only just beginning and we were discussing the change in the cosmetic market. We were uncertain at how hard Australia was going to get hit and where/what we would be doing day by day.

We were receiving at least 60+ phone calls a day from companies requesting urgent supply of packaging for mainly hand sanitiser bottles. We sold out of all stock we had available very fast and there were delays on re-supply for mainly bottles, pumps and sprays.

However it now seems that Australia has somewhat managed quite well to reduce the spread and flatten the curve which has now led to easing of some of these restrictions. At the time of writing Australia has been given a 3 step plan with dates on when/what restrictions will be eased (subject to the state/territory), which has given most brands piece of mind and a shift of focus on their projects. Since this has happened we have noticed a change in the market with current projects that were previously put on hold during the peak of the crisis, starting to resume.

In the due course we also expect to see a change in the cosmetic industry:

**1** *Brands will want to start working with local packaging suppliers rather than direct to offshore factories*

A lot of brands who went direct to China noticed long lead times and high price increases during the peak of the pandemic. The Australian dollar also dropped which led to packaging costing much more than they did originally. We are already working on major import replacement projects.

**2** *Brands will start holding more stock of their packaging*

With many people working from home and a huge demand for hand sanitiser packaging, lead times increased to 12-16 weeks. We expect more brands to hold more inventory so that they don't face the issue of being out of stock. We expect this to be the case for at least the rest of 2020.

**3** *There will be an influx in hand care/moisturisers post COVID-19*

With many healthcare workers as well as your average consumer using hand sanitiser multiple times a day, the alcohol in the product will irritate the skin. We expect there to be more skincare related products directed at this category.

Packaging supply has almost returned to normal aside from a few exceptions. Lotion pumps, fine mist sprays & foaming pumps are in worldwide demand, with lead times expected to be at a minimum September 2020. Unfortunately Australia is a very small market, most supply is currently going to Europe and the US. However at Weltrade Packaging we have close relationships with our suppliers and we are constantly bringing in sea containers of packaging every week.

Just in the last two weeks we have seen brands get moving nationwide, they are looking for something to put a new message out to the market that won't get lost in the Covid-19 news. Biodegradable packaging is one standout. It's been great to see the number of brands get on board already and to see their packaging hitting the shelves in the second part of this year will be fantastic.

It will really be a half glass full type of year, either worry about what has happened or look at the bright side and how so far as a country we have done well and move forward.

Our team is ready to talk about the next things that are happening in our industry, so pick up the phone and give us a call or send us an email. There is no better time to start a conversion. Speak soon and stay safe.

*Regards,* **Steve Welsh**

# the chemists and non-chemists guide to the basic science behind skin hygiene

by Wendy Free

Germs can and do cause disease, but is that anti-bacterial hand wash what you should be reaching for at present?

Every antimicrobial product, be it natural or synthetic works be attacking the weaknesses its' target.

Safe and effective antimicrobial products exploit the differences between us and the germs that can make us unwell. It's a balancing act, and recently the balance has changed … And we're now very focused on stopping or slowing disease transmission from a new virus …

*analysis of 22 studies reveals that human coronaviruses such as Severe Acute Respiratory Syndrome (SARS) coronavirus, Middle East Respiratory Syndrome (MERS) coronavirus or endemic human coronaviruses (HCoV) can persist on inanimate surfaces like metal, glass or plastic for up to 9 days, but can be efficiently inactivated by surface disinfection procedures with 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within one minute. Other biocidal agents such as 0.05–0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate are less effective. As no specific therapies are available for SARS-CoV-2, early containment and prevention of further spread will be crucial to stop the ongoing outbreak and to control this novel infectious thread*[1].

There are lots of different microbes, (micro-organisms/germs) that share our world with us, different types can and do include

- Yeasts & Moulds (Fungi)
- Bacteria Gram Positive, Gram negative
- Viruses (DNA, RNA enveloped, non-enveloped)
- Prions

In much the same way that we are different to trees, these types of living things are different to each other and to us. Here are some (very) basics.

## Animals

- Complex arrangements of specialised cells, mostly all interdependent on each other for functionality of the overall organism.
- Can have internal structural components like bones, muscles, or external ones (think insects)
- Gain energy by consuming other living things
- Breathes oxygen
- Reproduce via sexual reproduction (you'll see where I'm getting with this shortly),
- Made up of water, proteins, fats, carbohydrates and a little bit of DNA/RNA

## Yeasts & Moulds

- Can be single cells or grouped cells, cells have biochemical complex structures
- Gains energy by consuming other living things
- Many can swap between breathing oxygen to no-oxygen
- Reproduces by doubling in size and then dividing into two new ones.
- Made up of water, proteins, fats, carbohydrates and a little bit of DNA/RNA

### Bacteria

- Individual, self-sufficient, low complexity cells
- Classified according to their cell wall composition
- Can live in high or low oxygen, some can swap around
- Some digest biological materials, some convert light or chemicals to energy
- All reproduce by doubling and dividing
- Made up of water, proteins, fats, carbohydrates and a little bit of DNA/RNA

⋆anti-bacterial washes can effective against some bacteria; not all bacteria and not against other types of microbes⋆

### Viruses

- Don't have cells
- Only active, viable, reproducing when inside a living cell of another organism
- Use the resources of the living cell for energy and to reproduce, often times killing that cell in the process.
- Comprised of mainly protein, some have DNA, some have RNA, some have lipid layer

### Prions

- Don't have cells
- Only active, viable, reproducing when inside a living cell of another organism
- Use the resources of the living cell for energy and to reproduce, often times killing that cell in the process.
- Compromised of protein

So as you can see, the underlying commonality is protein and there after lipids (and RNA or DNA but that's locked away inside the cell); after that its cells.

So any antimicrobial substance will need to target, protein, lipids and/or cells, depending on the type of 'creature' it's trying to kill … Possibly it's obvious at this stage that products that are "antibacterial" might not be effective against viruses too? Many anti-bacterial substances work by limiting the reproductive effects of the bacteria, so this will have very little impact on viruses that reproduce very differently.

The World Health Organisation, National governments, State governments, Doctors, Nurses and Pharmacist all tell us that the BEST way to stop disease transmission is to wash our hands with **SOAP and water**. But have we forgotten what soap is?

*Actual historical records show soap-like materials in use by Sumerians in 2500BC and there are references to soap in Greek and Roman records and by the Celts in northern Europe. As European civilizations emerged from the Dark Ages in the 9th and 10th centuries soap making was well established and centered in Marseilles (France), Savona (Italy), and Castilla (Spain). In those days soap was a luxury affordable only by the very rich. Mass manufacture of soap started in the 19th century and was well established by the turn of the century with individually wrapped and branded bars*[2].

## What is soap?

a) Stuff the makes you cleaner
b) Anything labelled as soap
c) A bar of something that foams when wet and lathered
d) A liquid that kills germs
e) All of the above

Not necessarily … **Soap has a legal meaning** included in a number of legislative instruments which usually end up as something like *"a preparation derived from the action of a solution of alkali on fats or oils of animal or vegetable origin"*.

The oils used in soap making are mostly triglycerides; when they are treated with lye and/or caustic soda they hydrolyse to the fatty acid salts (the soap) and glycerol.

- Soap is (usually) the sodium salt of a fatty acid, (although there are potassium variants).
- C12–14 soaps are soluble and lather easily.
- C16–18 soaps are less soluble but good for forming solid bars.

Soap (alkyl carboxylate) and syndet ("synthetic detergents" ie 'beauty bars' aka acyl isethionate) are anionic surfactants and like all anionic surfactants they interact with skin proteins and skin lipids.

- With soap, the carboxylate head group is compact; it has a high charge density that facilitates enhanced binding to and denaturation of proteins.
- In the milder synthetic versions, the isethionate head group is larger and more diffuse, its mildness is a consequence of a low charge density and a lesser ability to interact with proteins.

The pH of soap is typically in the pH range 9–11.

The high pH temporarily disrupts the pH dependant processes of the stratum corneum (skin), as well as negatively impacting the proteins, fats and carbohydrate structures of the microbes that reside on or are transient on the skin surface. Above pH ~8 proteins swell, distorting their structural integrity and the lipid bilayers that make up the envelope that surrounds bacteria and some viruses begins to saponify, rapidly killing these microbes. Our multilayered skin is far more tolerant of these chemical challenges than are the germs that might be there.

Soap (and water) can work best because

- It acts firstly against fats on the skin that can coat, hide, protect the germs from outside forces
- It works against proteins that make up the structural and functional germs using both pH and chemical attack
- It also works against the fats that can provide structure support to some of the viruses and bacteria
- The water physically removes the germs from the skin surface, including any that might have been sub-lethally dosed and helps with protein dissolution.
- Physical drying with a clean towel also helps to physically remove germs; hot air drier may just blow germs back on to the skin.

## Alcohol based sanitisers

Alcohol based sanitisers dissolve proteins. They do not work well with fats, in fact if you have oil on your hands, this can protect the germs that are there and also reduce the effectiveness of

the alcohol. Proteins don't dissolve in pure alcohol, they need a bit of water there to help, that's why sanitisers will have between 62% and 90% ethanol or isopropanol, as well as water.

*Alcohol solutions containing 60–80% alcohol are most effective, **with higher concentrations being less potent**. This paradox results from the fact that proteins are not denatured easily in the absence of water*[3].

*Alcohols have excellent in vitro (ie lab based) germicidal activity against Gram-positive and Gram-negative vegetative (growing) bacteria (including multidrug-resistant pathogens such as MRSA and VRE), M. tuberculosis, and a variety of fungi. However, they have virtually no activity against bacterial spores or protozoan oocysts, and very poor activity against some non-enveloped (non-lipophilic) viruses*[4].

Some people add glycerol or propylene glycol to sanitiser to reduce the drying on the skin, this is unlikely to effect the action on germs, but adding aloe vera, or other skin conditioners might actually reduce the effectiveness, so BE CAREFUL in doing this. Please also think about what else you are adding, particularly the purity of any amines!

Alcohol based sanitisers are not magic, they need time to work.

*Typically, log reductions of the release of test bacteria from artificially contaminated hands average 3.5 log10 ($10^{3.5}$) after a 30-second application, and 4.0–5.0 log10 ($10^{4-5}$) after a 1-minute application*[5].

## What does kills 99.99% of germs mean?

WARNING SCIENCE CONTENT – you'll be sorry you asked … (but good practice for me to explain it)

"Real maths"
1 = 1
10 = "10 to the power of 1" or $10^1$
100 = $10^2$
1,000 = $10^3$
10,000 = $10^4$
100,000 = $10^5$

so in real numbers $10^{3.5}$ to is about 3162

"Microbiology" testing is in an imprecise science, *it's always an estimate,*
1 = less than 2
10 = less than 20
100 = less than 200
1,000 = less than 2,000
10,000 = less than 20,000
100,000 = less than 200,000

Testing kill rates looks at reductions in the population so it depends on what you start with.

Say you start with 1,000,000 ($10^6$) which is a pretty typical number; if the product gives you a 3.5 log reduction (3.5 orders of magnitude) you end up with (1,000,000 => 100,000 => 10,000 => 1,000 =>) ~684 = 99.94% kill rate (standard maths), but if you add in test uncertainty, you end up with a value of "less than 1368", which gives a kill rate of 99.86%. So with anti-microbial testing you always want *at least* a log 4 reduction … after all those remaining bugs can double in minutes under the right conditions.

## Anti-bacterial vs Anti-viral

Cosmetic (ie non-TGA products) can claim antibacterial, and, as of recently, also 'sanitising'; as soon as you claim anti-fungal, anti-viral or name a disease, these products are TGA.

Regardless of the claim, you need suitable evidence to support your claim.

- EN 1499 – hygienic hand wash tests the product against *E.coli* a bacteria.
- EN E-1174 – tests using two bacteria, S. marcescens and *E. coli*.
- ASTM E-1838 looks for efficacy against some viruses such as Adenovirus, rotavirus, rhinovirus and hep. A
- TGA also has specific requirements[6], especially when it comes to 'you know who'…

Please don't be fooled into thinking that 'antibacterial' hand washes will be as effective against some of the more nasty germs that cause disease, all in all, it's probably time to go back to good old soap and water, so long as it's real soap!

## References


1 Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents J Hosp Infect. 2020 Mar; 104(3): 246–251. Published online 2020 Feb 6. doi: 10.1016/j.jhin.2020.01.022

2 Cosmetic Dermatology, Products and Procedures Zoe Diana Draelos MD, ISBN 978-1-4051-8635-3

3 WHO Guidelines on Hand Hygiene in Health Care, 2009 ISBN 978 92 4 159790 6

4 WHO Guidelines on Hand Hygiene in Health Care, 2009 ISBN 978 92 4 159790 6

5 WHO Guidelines on Hand Hygiene in Health Care, 2009 ISBN 978 92 4 159790 6

6 https://www.tga.gov.au/otc-medicine-monograph-hand-sanitisers


Stay well,

Please feel free to contact me, obligation free at any time,


Wendy Free B.Sc M.Tech Mngt MASM MRACI FAOQ

Quality Matters Safety Matters Pty Ltd.

# sunscreen highlights

by John Staton

# Two down, One to go!

With the publication of the new ISO standard for water resistance test – ISO 16217[1] this month, only one of the "set" of three standards cross referenced by the Australian/New Zealand Standard 2604 [2] is now pending. This is the method which measure broad spectrum and is only under minor review. The timing for this is set for release late 2020 and so the AS/NZS 2604 update is being readied for public comment, with publication before year end.

The major changes that will appear in the updated AS/NZS 2604 will be the replacement of the section related to water resistance with ISO 16217.

As I have indicated previously, there should not be any issue of needing to retest for compliance to the new standard for sunscreen products which are already in the market. Previously, TGA had taken the position that retesting was a good practice for products coming up to 10 years in the market.

## Remote Control ISO Meetings

In line with most of the world, ISO meetings are currently being conducted on line. The next round is due over the week of 15th to 18th June. The hottest topic under discussion is the progress of in vitro SPF test methods – ISO 23675 [3] and ISO 23698 [4]. Each of these proposes a different approach to an alternative to in vivo testing. As most sunscreen test labs have been closed or are severely restricted in the conduct of testing over the last few months, these new methods are a hope for at least use as a screening test prior to more expensive testing involving the use of human test volunteers.

## USA Falling Further out of Step!

In February 2019, the FDA published its proposal to implement changes to the regulation of sunscreens in the USA [5], including some which impact on testing. While some of the issues highlighted by FDA revolve around validation and test conduct deficiencies in the US, several result in variances to what is now practiced in at least 60 countries of the World.

The first major variation relates to the definition of what we know in Australia as broad spectrum. For the USA, the now widely accepted UVAPF/SPF ratio of 1/3rd is replaced with a new FDA proposal [6] for UVA I/UV ratio of 0.7 or higher. See Fig 1.

Secondly, for SPF, the FDA now proposes to extend the highest label

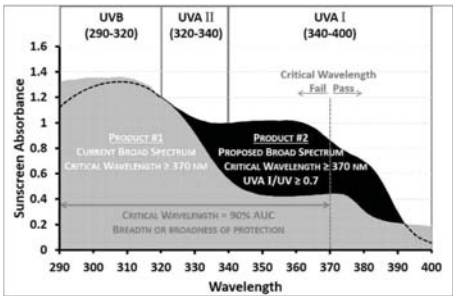



Fig 1. FDA guidance for Current versus Proposed Broad Spectrum.

category to 60+. In Australia, and most of the regulated parts of the world, this is currently set at 50+. Apart from the difficulty in achieving this higher SPF, limitation on the permitted content of UVA absorbers makes compliance quite a challenge.

## References


1. ISO 16217 – Cosmetics - Sun protection test methods – Water resistance – Water immersion procedure

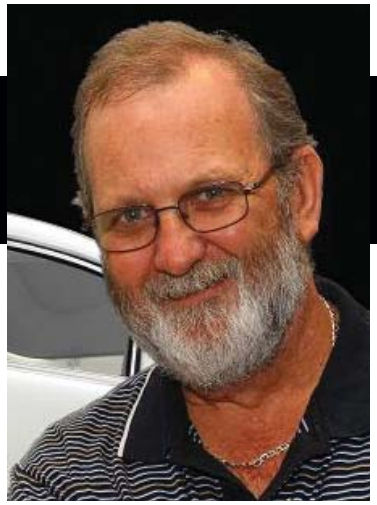
2. AS/NZS 2604 :2012 – Sunscreen products — Evaluation and classification

3. ISO 23675 Cosmetics — Sun Protection Test Methods – In Vitro Determination of Sun Protection Factor (SPF)

4. ISO 23698 – Cosmetics – Sun protection test methods – Measurement of the Sunscreen Efficacy by Diffuse Reflectance Spectroscopy"

5. Federal Register/Vol. 84 No. 38 /Tuesday February 26 2019

6. https://s3.amazonaws.com/images.federalregister.gov/EP26FE19.002/original.png?1550690433



## Part 52 –

by Ric Williams

# *pH, Acidity, Alkalinity*

pH is used in Chemistry as a symbol to indicate the acidity or alkalinity of a solution. It is a measurement of the hydrogen ion concentration and is expressed on a scale of 0 to 14.

In strict chemical terms the pH is defined as;

$pH = -\log_{10}([H+]) = \log_{10}(1 \div [H^+])$

where $[H^+]$ is the Hydrogen ion concentration or the amount of free Hydrogen ions in solution.

The most concentrated solution you can have is 1 in 1 or a pH of 0.0 (zero) and

The highest pH is where $[H^+]$ is at its lowest of 1 in $10^{14}$ or a pH of 14.0

**eg.** if the Hydrogen ion concentration is 1 in $10^5$ then pH = $-\log [1 \div 10^5] = 5.0$ whereas if the Hydrogen ion concentration is ten times that ie. 1 in $10^4$ then pH = $-\log [1 \div 10^4] = 4.0$

If the Hydrogen ion concentration is 1 in $10^{12}$ ie. 100,000 times less than in a neutral solution then pH = $-\log [1 \div 10^{12}] = 12.0$ – sometimes, when the solution is alkaline, we refer to pOH and in this case it would be a pOH of (14.0 – 12.0) = 2.0

as you can see this is a logarithmic value and this means that a pH of 6.0 is ten times the acidity of that of a pH of 7.0, 5.0 is ten times the acidity of that of a pH of 6.0, 4.0 is ten times the acidity of that of a pH of 5.0, etc.

Consequently, in the range 7.0 – 14.0 a pH of 8.0 is ten times the alkalinity of that of a pH of 7.0, 9.0 is ten times the alkalinity of that of a pH of 8.0, 10.0 is ten times the alkalinity of that of a pH of 9.0, etc.

A pH of 1 is very acidic reducing in acidity to pH 7 (which is considered neutral) and then increasing alkalinity to pH 14.

Note that pH depends on temperature. For instance at 0°C the pH of pure water is 7.47. At 25°C it's 7.00, and at 100°C it's 6.14.

## Neutralisation

is where you have an acidic medium and add base (alkali) to this to bring the pH from its low value up to pH 7. It also works in reverse where you have an alkaline medium and add acid to this to bring the pH from its high value down to pH 7. At this point the medium is considered "Neutralised".

If an acidic medium (say pH 3.0) is treated with an insufficient amount of alkali and the pH only is raised to 5.5

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This column is intended not only as an education tool for non-technical people or beginners in our industry, but as a forum for those wishing to enlighten all about recent technology advances and new ideas. I hope experienced scientists will also contribute to this ideal and if you wish to do so please email me at: ric@cosmepeutics.net.au and I will publish your comments.

then this is called partial neutralization, and vice versa for alkali mediums.

If an alkali medium (say pH 9.0) is treated with acid and the pH drops to pH 5.5 then this would not be called neutralisation but acidification. If an acidic medium is treated with alkali and the pH goes above pH 7.0 then this is called alkalination.

## Buffered

a stable mixture of weak acid and base which when put into solution or onto the skin reduces the effects of strong acids or bases. The pH does not change as much as would normally be expected and the solution is called buffered. It keeps the pH of skin within a small defined range avoiding dramatic changes which may cause skin damage.

## Buffering

is where certain salts are added to an acidic or alkaline medium to reduce the effect of adding acid or alkali. This occurs where the salt ions absorb added alkali or acid giving what appears to be less added acid or alkali.

Examples are;

0.05M Phosphate Buffer

3.40 grams of Potassium DiHydrogen Phosphate and 3.55 grams of DiSodium Hydrogen Phosphate dissolved in 1 litre of water will buffer solutions to a pH of 6.88

0.05M Potassium Hydrogen Phthalate Buffer

10.21 grams of Potassium Hydrogen Phthalate dissolved in 1 litre of water will buffer solutions to a pH of 4.00

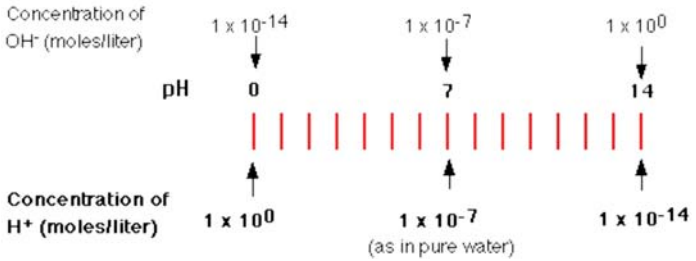
The most obvious areas where body solutions are buffered are Blood (where small changes in pH can be critical in the transfer of Oxygen to the cells), and the digestive system (where the stomach has a pH of about 2.0 to breakdown foods while the Pancreas and Bile Glands buffer this solution to a more neutral pH to allow better absorption of the broken down foods through the intestines, but of most interest to the cosmetic chemist or a beautician is the Acid Mantle.

## In Practice

The pH scale is traceable to a set of standard solutions whose pH is established by international agreement.[3] Primary pH standard values are determined using a concentration cell with transference, by measuring the potential difference between a hydrogen electrode and a standard electrode such as the silver chloride electrode. The pH of aqueous solutions can be measured with a glass electrode and a pH meter, or a color-changing indicator. Measurements of pH are important in chemistry, agronomy, medicine, water treatment, and many other applications.

At 25°C, solutions with a pH less than 7 are acidic, and solutions with a pH greater than 7 are basic. The neutral value of the pH depends on the temperature, being lower than 7 if the temperature increases. The pH value can be less than 0 for very strong acids, or greater than 14 for very strong bases.[2]

Is pH important? **Yes!**




The properties of most proteins, enzymes for example, are sensitive to pH.

## As the pH drops,

- $H^+$ bind to the carboxyl groups (COO-) of amino acids aspartic acid (Asp) and glutamic acid (Glu), neutralizing their negative charge, and
- $H^+$ bind to the unoccupied pair of electrons on the N atom of the amino ($NH_2$ ) groups of amino acids lysine (Lys) and arginine (Arg) giving them a positive charge.

**The result:** Not only does the net charge on the molecule change (it becomes more positive) but many of the opportunities that its R groups have for ionic (electrostatic) interactions with other molecules and ions are altered.

## As the pH rises,

- $H^+$ are removed from the COOH groups of Asp and Glu, giving them a negative charge (COO-), and
- $H^+$ are removed from the NH3+ groups of Lys and Arg removing their positive charge.

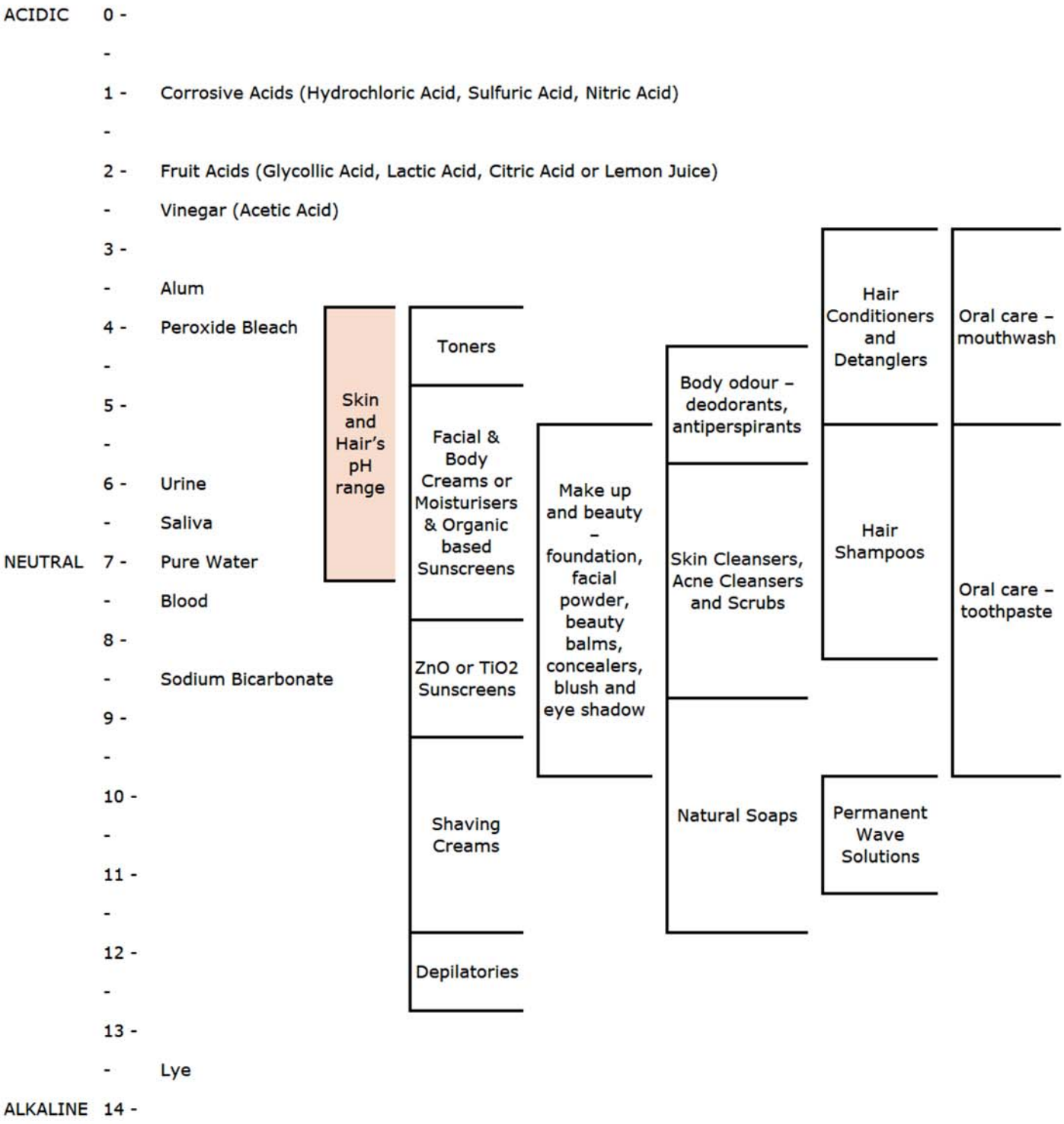
**The result:** Again the net charge on the molecule changes (it becomes more negative) and, again, many of the opportunities its R groups have for electrostatic interactions with other molecules or ions are altered.

The pH of the **cytosol** within a human cell is about 7.4. BUT, this value masks the pH differences that are found in various compartments within the cell. For example,

- The interior of **lysosomes** is much more acidic (as low as pH 4) than the cytosol, and the enzymes within work best at these low pH values.
- The pH differential created within **chloroplasts** by the energy of the sun is harnessed to synthesize ATP which, in turn, powers the synthesis of food.
- The pH differential created within **mitochondria** during the respiration of food is harnessed to the synthesis of ATP which, in turn, powers most of the energy-consuming activities of the cell such as locomotion and biosynthesis of cell components.

The pH of seawater is typically limited to a range between 7.5 and 8.4. It plays an important role in the ocean's carbon cycle, and there is evidence of ongoing ocean acidification caused by carbon dioxide emissions. However, pH measurement is complicated by the chemical properties of seawater, and several distinct pH scales exist in chemical oceanography.

## *The pH Scale*




Products with little or no water do not have a measurable/meaningful pH. Neither do products with high Ethanol/Glycol content.

eg Hair care – hair dye, hair styling products

Body odour care – hygiene powders

Skin care – lip balms, , hand and feet emollients, , face masks

Make up and beauty – facial powder, blush and eye shadow, mascara, lip and eye liners, nail polish

Hair removal – depilatory waxes

Scents – perfumes and perfumed sprays

Some baby care – powders, wipes

## Explanations of items in the pH Scale chart

Skin and Hair's pH range is usually between 4.0 and 7.0, due to the varying content of fatty acids contained in sebum and sweat.

High levels of fatty acids is due to very oily skin and can result in a skin pH as low as pH 4.0.

Oily skin is the result of the overproduction of sebum from sebaceous glands. These glands are located under the skin's surface. Too much sebum, can lead to clogged pores and acne.

Very dry, or Alipoid, skin can have a pH as high as 7.0 due to the absence of fatty acids.

It is considered that normal skin, has a good balance of fatty acids and will have a pH of approximately 5.6

Skin Toners were originally developed to remove residual soap from skin when alkaline soaps were the primary mode of skin cleansing. Hence, they had a low pH to counteract the alkalinity of the soap and were dilute solutions of alcohol or other solvent for soaps. With the advent of neutral cleansers that are highly water-soluble modern toners have taken on other purposes, such as skin conditioning.

Facial & Body Creams or Moisturisers & Organic based Sunscreens can have a wide range of pH values, mainly due to marketing claims, but there is a basic underlying theory on which pH your moisturiser or face cream should have.

a Facial & Body Creams or Moisturisers & Organic based Sunscreens for oily skin, apart from only having low levels of neutral oils and no fatty acids, should have a pH in the neutral to slightly alkaline range (pH 6.5 to 8.0) to combat the acidity from the high levels of fatty acids in oily skin.

b Facial & Body Creams or Moisturisers & Organic based Sunscreens for dry skin, apart from only having higher levels of fatty acids, should have a pH in the neutral to acidic range (pH 6.5 to 4.0) to increase the acidity in dry skin.

c Facial & Body Creams or Moisturisers & Organic based Sunscreens for normal skin, despite what marketing hype is out there, needs to have a pH that will not affect its normal acidity/alkalinity (ie approx. 5.6). That is, the pH of these products should be approximately neutral (pH 6.5 to 7.5) as if it an acidic skin cream (pH 5.5) on skin with a pH of 5.5 this would be increase the overall acidity, possibly causing oily skin as a result.

Sunscreens containing ZnO or TiO2 need to be slightly alkaline, as an acidic skin cream would dissolve the alkaline earth minerals (ZnO or TiO2) causing a loss in efficacy over time.

Shaving creams based on soaps need to be alkaline for a few reasons;

a Soaps need to be alkaline for stability of the soap.

b The alkaline soaps have a softening effect on facial or other hair, making removal easier.

Depilatories have a high pH as, at this pH, they tend to open hair cuticles and dissolve the hair strand.

Make up and beauty – foundation, facial powder, beauty balms, concealers, blush and eye shadow, that contain minerals (mineral makeup) need to be slightly alkaline, as an acidic skin cream would dissolve the alkaline earth minerals (eg Kaolin, Clay or Talc) causing a loss in efficacy over time.
If the product does not contain alkaline earth minerals then a lower pH is possible.

Deodorants, and in particular antiperspirants, usually have a low pH to improve antibacterial properties however higher pH products are also found.

Skin Cleansers, Acne Cleansers and Scrubs, based on synthetic surfactant solutions, can have a wide range of pH values, the major consideration being not to have a pH too low where irritation might increase or the particular surfactant will hydrolyse (dissociate), in solution, causing instability and rancidity. These problems must be sorted out with longer term stability trials of skin testing.

Natural Soaps are those based on the saponification of fats or fatty acids, with alkaline solutions, such as Sodium Hydroxide solution (Lye).

(ie Fat(triglyceride)+Alkali=Soap+Glycerin or Fatty Acid+Alkali=Soap+Water).

An old fashioned soap bar can have a pH in the range 9.5 to 11.5, with the former have post added fatty acids (superfatting) and the latter having other additives eg Sunlight soap, baby soap, castile soap or carbolic soap.

Clear soaps (eg Neutrogena cleansing bar) is made from fatty acids saponified with amines (eg Triethanolamine and a small amount of Potassium Hydroxide for hardness).

In later years "Beauty Bars" (eg Dove), based on synthetic surfactants mixed with hardeners have dominated and the pH of these can be in the range of pH 5.0 to 7.0. Note; they are called "Beauty Bars" or "Cleansing Bars" as they generally do not contain sufficient soap of qualify as a soap under the Australian Standard.

Hair shampoos were originally based on soaps but converted to synthetic surfactant blends as these were less harsh on the hair and can have a pH in the range of pH 5.0 to 7.0. Slightly alkaline shampoos would be suited to oily hair problems.
The major consideration being not to have a pH too low where irritation might increase or the particular surfactant will hydrolyse (dissociate), in solution, causing instability and rancidity. These problems must be sorted out with longer term stability trials of skin testing.

Hair Conditioners were originally designed to counteract the harsh effects of soap-based shampoos. The cationic surfactant reacted with the soap (causing it to come out of solution and be washed away) and the low pH counteracted the higher pH of soap.

With the advent of synthetic surfactant-based shampoos, the cationic surfactant reacted with the synthetic surfactant (causing it to come out of solution and be washed away) and the low pH counteracted the higher pH of the shampoo. The lower pH also had the benefit of closing the cuticles increasing smoothness (shine) and reducing combing drag.

Permanent wave solutions were designed to break to Cysteine-Cysteine bonds that held the shape of the hair strand (due to its high alkalinity) and when the hair was "neutralized" it allowed the hair strand to reform in a new configuration (usually held by rollers).

Mouthwashes (and breath fresheners) were generally acidic for mouth freshness and to increase the antimicrobial activity of Cetyl Pyridinium Chloride which was used as the major surfactant. High levels of alcohol and essential oils/thymol also did this and as stated above, solutions with high levels of alcohol cannot have a meaningful pH value when measured.

Toothpastes had a pH dependent on the type of abrasive used. When Dicalcium Phosphate was used a higher pH is obtained, Similarly Sodium Bicarbonate or Calcium Carbonate caused higher pH's. Slight acidic pH can be obtained with Silica based toothpaste. Note Silica based toothpastes were used in clear toothpaste formulations, as other abrasives mentioned are opaque in suspension.

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# Patenting Opportunities in the Fragrance Industry

**by Dr. Jim Onishi MRACI C.Chem** and **Dr. Elizabeth Houlihan FRACI C.Chem**

Houlihan[2] Patent & Trade Mark Attorneys

www.houlihan2.com

It is well known that the fragrance industry is big business. Annual sales from each of the four main global fragrance producers are in the billions of dollars. The intellectual property invested in commercial fragrances is extensively protected.

## How does the fragrance industry protect its intellectual property?

Traditionally, industry secrecy was the main form of protecting proprietary information. The proprietary information included, for example, key fragrance compounds (known as 'captives'), formulations, essential oil extraction processes, and analytical techniques. Use of trade secrets is still an adequate form of intellectual property protection in the fragrance industry, provided that the 'secret' proprietary information can be identified, and suitable safeguards are put in place to block secrets from getting out to competitors. The reality is that misappropriation of proprietary information is a real threat because frequent job and career changes within a person's life is now the norm. Once proprietary information is exposed, establishing misappropriation can be a challenge, if action against the perpetrator is to be pursued. Worse still, after court proceedings are instituted, the difficulty lies in revealing the proprietary information itself, to the public, during the proceedings. Trade secrets alone can no longer constitute an adequate form of protection.

Patents are an alternative form of intellectual property protection, which are available to protect proprietary information. Patents grant the patent owner an exclusionary right for a general term of up to 20 years from the date of filing. A key advantage of patents is that they are enforceable, without identifying the specific proprietary information being misappropriated by a former employee (or his/her employer), provided that the infringement falls within the scope of the patent claims. Patents can therefore be a useful tool to protect proprietary information.

Patents can be obtained for any man-made innovations. It should be noted that in some countries, such as the US, naturally occurring compounds cannot be patented. Fortunately, at least in Australia, isolated forms of the naturally occurring compounds can be patented, as long as they are not perceived as constituting 'information', such as genetic information that is incorporated into the isolated nucleic acid sequence. Patents can also be obtained for novel and inventive synthetic pathways for making natural products, as well as for any non-naturally occurring derivative compounds of natural products. Formulations comprising at least one novel and inventive component in the formulation would also be patentable. If the components are known, the formulation may nevertheless be patentable if the components interact in a manner in which they provide a new and non-obvious characteristic or property.

## Perfume formulations

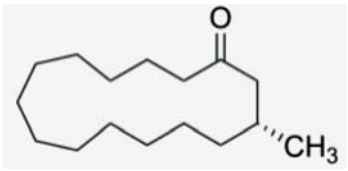
Perfume formulations are fascinating in that they allow the re-creation of fragrances found in natural environments. They also allow the creation of new pleasant fragrances not found in nature. Identification of captives isolated from nature and investigating their properties, including any of their synthetic derivatives, may yield the discovery of new pleasant fragrances, unique characteristics or new applications in perfume formulations. High rewards are therefore possible in this billion-dollar industry.

Modern perfumes comprise three

parts: a top note, a heart note and a base note. The top note is the first impression of the scent. Due to the high volatility of these small and lightweight captives, they provide the immediate smell upon application to skin. The top note usually mimics leafy green and fruity fragrances. The heart note is the main scent and comprises heavier and less volatile captives. The heart note usually mimics flower fragrances, such as jasmine, lily, cherry blossom and the like. The base note is the last scent to emerge and involves the heaviest captives. These are the least volatile and remain on the skin the longest. The base note usually mimics vanilla, musk, precious woods and the like. Beyond these 'notes', the formulations would include surfactants to make the captives water-soluble and to reduce their surface tension, making the various components miscible in the formulation. Additives in the form of colourants, stabilisers (such as chelating agents and UV absorbers) and antioxidants are often included. The main component of all perfume formulation is the solvent medium, usually distilled water and ethanol, which assists with dispersing the fragrance over the skin.

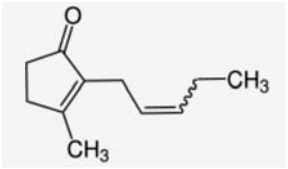
## Naturally occurring compounds

Naturally occurring compounds in perfume may include jasmone from jasmine flowers, civetone from civet cats and/or muscone from musk deer. Natural muscone is no longer used because it is isolated from the glands of musk deer, which is an endangered species. The synthetic alternative, l-muscone, can be used, but is expensive. Fortunately, only a small amount of synthetic muscone gives a musk effect, comparable to that of the natural product.



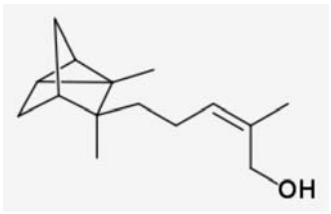

l-muscone

Jasmone can be extracted from a natural source or synthesised. Interestingly, synthetic jasmone has a stronger odour than its natural counterpart. This means that the synthetic version can be used sparingly, thereby reducing manufacturing costs, therefore making the resulting perfume more ecologically friendly.

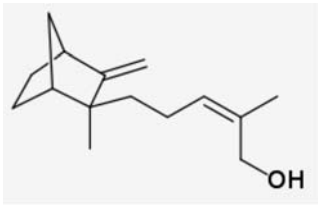



Jasmone

For woody fragrances, natural sources of sandalwood oil may be used. These can be derived from East Indian sandalwood, West Indian sandalwood, African sandalwood or West Australian sandalwood. Of these, the East Indian sandalwood is most prized, because of its high α-santalol and β-santalol content and composition consistency. However, due to extensive deforestation and environmental concerns, Indian sandalwood is now declared an endangered species.



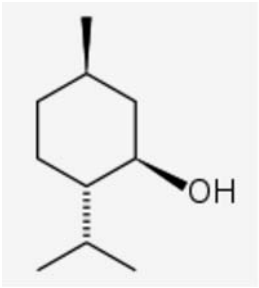

α-santalol




β-santalol

While modern perfumes use a combination of natural and synthetic ingredients, where the natural ingredients are often cultivated in developing countries, one problem with natural ingredients is in maintaining consistency of the quality of the ingredient, which may vary as a result of annual variations in climate-cultivating conditions.

In the search for innovative nature-inspired synthetic derivatives, researchers have identified that moving the location of substituents or bonds within the captive can have a drastic effect on smell. For example,  -terpene has a lemon-like odour whereas terpinolene, its isomer, has a woody-pine odour. Menthol is another useful component in fragrances. The natural product is primarily one stereoisomer, shown here.




(1R,2S,5R)-(–)- menthol

Chiral-catalysed hydrogenation reactions have been used for the chiral synthesis of menthol and other fragrance compounds. The use of these reactions has shown that chiral compounds have different fragrances.

The identification of the signature fragrance molecule associated with a target smell of interest can be done relatively easily using current chemical analytical technologies and chemical synthetic techniques. The conventional technique in the industry involves using headspace analysis to sample volatile chemicals, followed by isolating and identifying each molecule through GC-MS. This technique has made reverse engineering of fragrances faster, cheaper and simpler.

## What opportunities might be available?

As noted, there is a need for new smells and smell-alike compounds in the fragrance industry. This, in part, would support the conservation of endangered species and minimise environmental impact of natural habitats of wildlife. We would like to encourage researchers to re-visit their laboratory notebooks and identify whether any compounds made in the course of their past research

projects possess a scent. If the scent is woody or flowery, that compound may be a stepping stone to the discovery of a potentially useful new captive. Perhaps the newly identified captive has a more potent aroma, or can be synthesised cheaply? If the newly identified captive is safer and less allergenic than the natural product, then it would be even more valuable.

Patents could be pursued for protecting these newly identified captives, for their synthesis pathway (i.e. methods of making the captives), and perfume formulations involving the new captives. Ideally, patents should be pursued on all these forms to plant a thicket of patents to be used as obstacles to deter competitors. However, in reality, pursuing all these forms individually in a patent can become very expensive. The best pathway forward would depend on the goal to be achieved. For example, if you are a scientist employed by a research institute or a university, a patent owner may monetise its patent by selling it, or licensing it to interested parties and then collecting royalties from them. Patents are therefore valuable commodities.

## Do you have something worth patenting?

It may be prudent to consult with a patent attorney before you file that old lab notebook away, just in case you have inadvertently synthesised something worth patenting. Perhaps a short remark made about a woody smell scribbled on a corner of your notebook page, previously overlooked, may lead to a future, valuable practical use in the fragrance industry.

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# Antimicrobial testing: what do the results mean?

by Kevin Roden


## Abstract

Tests designed to determine the antimicrobial efficacy of a product, such as a preservative in a cosmetic, a sanitiser in a household cleaner, or even a disinfectant, have set pass requirements. These often specify minimum log reductions or % kill to meet the requirements of the test. This paper will discuss how these tests are conducted, what these terms mean, how they are calculated and how changes in the method used for the testing may lead to variations in the results achieved.

The presentation will also cover changes to the Laboratory Accreditation Standard, ISO 17025 with regards to Measurement of Uncertainty and Statements of Conformity and what impact these changes may have on certificates of analysis issued for Preservative Efficacy Testing.


## Antimicrobial Testing: what do the results mean?

When any product is formulated with antimicrobial active substances, such as preservatives or biocides, both referred to as biocides in this paper, there may be interactions between the biocide and the product or container. The biocide may be lost due to partitioning into the oil phase or micelles, interaction with other ingredients, absorption onto the closure or container or adsorption onto solid particles. These factors may reduce the level of biocide available leaving only the aqueous phase residual concentration. This may be further compromised by converting to an inactive form due to a pH effect or simply be degraded.

It is difficult to determine the aqueous phase residual concentration by chemical analysis as extraction techniques may recover bound, absorbed or adsorbed active and give false high results. Microbiological testing is a convenient way to determine the amount of active material available in the water phase, as this is where microorganisms are found.

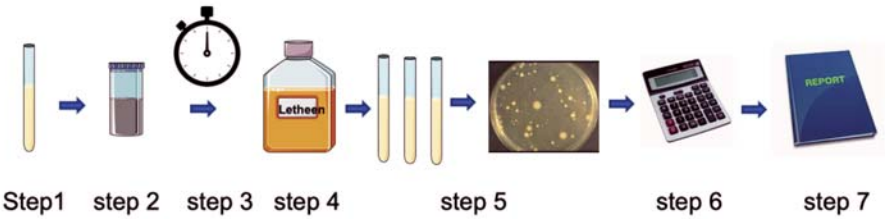
Microbiological Testing for antimicrobial activity follows a simple pattern as shown in Table 1 and Diagram 1. Once an appropriate test method is selected all tests follow the same basic format.

**Table 1 Steps in Antimicrobial testing**

| Step | Procedure |
|---|---|
| 1 | Prepare the test organisms |
| 2 | Add test organisms to the product under test |
| 3 | Allow a desired contact period |
| 4 | Neutralise the antimicrobial |
| 5 | Determine survivors |
| 6 | Calculate Log reduction or % kill |
| 7 | Report the results |

Although the basic procedure is fairly simple to conduct, variations in the procedure can result in significant changes to the results achieved. Published standard test methods usually specify the steps to be taken to ensure reproducibility of the results. Possible variations in all steps from 1 – 6 may

Diagram 1. Steps in Antimicrobial Testing



affect the results obtained and what they actually mean.

## Step 1: Preparation of the test organism.

The test organism plays an obvious and critical role in the test outcome. The species of microorganisms used in the test must be selected on the basis that it is realistic, that is, that they would need to be controlled by the material under test. Cosmetic preservatives are tested with a selection of organisms to cover the range of Gram positive and Gram negative bacteria as well as a yeast and a mould. The species selected have either been isolated from infected cosmetics or are expected to be placed into cosmetics either during production or by the consumer. Likewise, the bacteria selected to test antibacterial hand wash, typically *St. aureus* and *E. coli* or a *Klebsiella sp.* on the basis that they are either skin or gut commensals and would expect to be encountered on hands.

All microorganisms exhibit an intrinsic tolerance to biocides and it is the naturally chromosomal controlled property or adaption of an organism. This may be expressed by a number of factors, including morphology, biofilm formation, nutritional starvation or growth rate control.

Bacteria are divided into two groups: Gram positive and Gram negative, based on the ability of a dye to bind to the cells. Gram negative bacteria possess an additional outer membrane, which stops the dye binding to the cell and also makes them tolerant to higher concentrations of biocide than Gram positive bacteria. The outer membrane acts as a further permeability barrier to entry of biocides, many of which must penetrate the cell membranes to reach their targets within the cytoplasm of the cell.

Pseudomonads are commonly more tolerant to biocides than most other microorganisms. They have been found to have differences in their outer membrane permeability of up to 400 times that of other Gram negative organisms, making it more difficult for biocides to enter. [1,2]

The strain chosen is not as important, provided the same strain is used by all laboratories conducting the test as variability in susceptibility occurs across any population. The maintenance of cultures is paramount in keeping the culture homogeneous in this respect. Continuous subculturing with inherent mutation/variation can result in a culture that behaves quite differently to the parent stock and care must be taken to minimise passage numbers or generations away from the original culture held in a reference collection.

Diagram 2. Development of MIC Variation in a Population

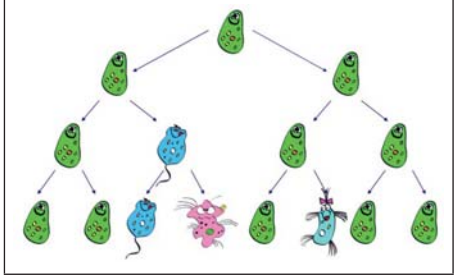
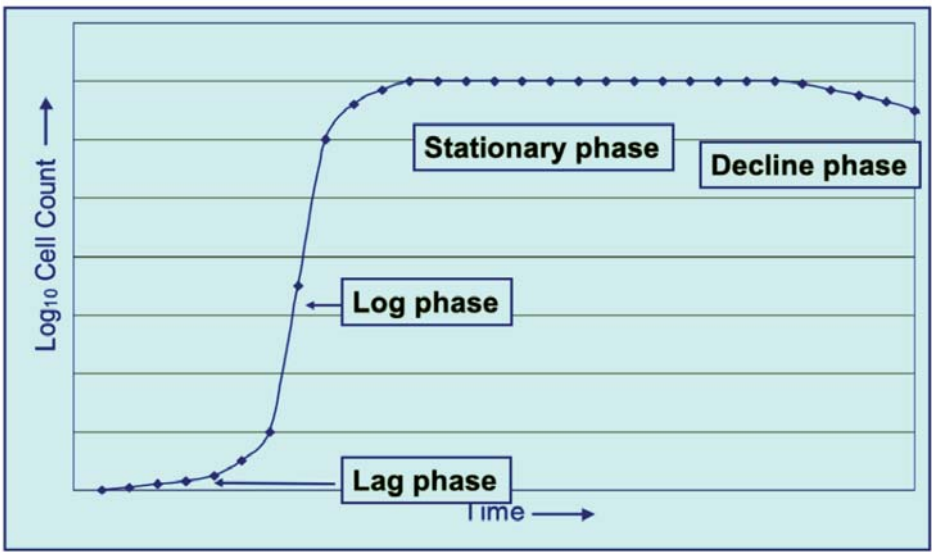


Diagram 2 illustrates natural variation that occurs across generations resulting in offspring with varying tolerance to biocides. Obviously, the more generations the greater the potential for variations to occur. However, if factory isolates known to express tolerance to the biocide are a concern then they should be used in addition to or replace the usual culture collection organism. Special care must be taken to ensure the tolerance is not lost by the cultured organisms as this can rapidly occur. It must also be considered that the use of these special organisms in conducting tests will require higher levels of biocides to pass the test requirements than that required by usual test organisms.

The effect of this variation in the population may affect test results. A population with a wide variation in the Minimal Inhibitory Concentration (MIC) against a biocide will likely show a result with a fast initial kill due the higher number of cells showing a low MIC value followed by a slower and less complete kill due to the number of more tolerant organisms. In contrast a population that has a narrow variation on MIC values will likely show slower initial action followed by a rapid and complete kill.

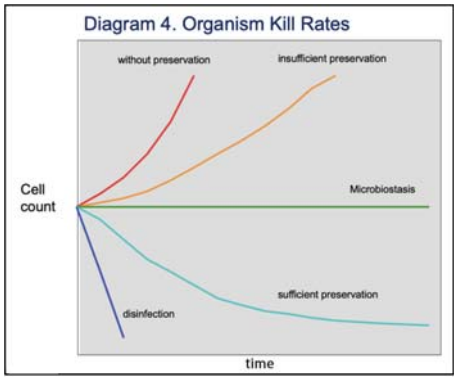
The growth phase of the culture also determines the tolerance to biocides. The Growth curve shown as diagram 3 shows the 4 phases of growth of any population. Test organisms are usually prepared in either log (exponential growth) or stationary phase and the age of the culture determines this. Standard Test methods usually stipulate the period of incubation of a test culture to ensure it is in the appropriate growth phase. Cultured bacterial cells supplied with excess nutrients grow quickly during exponential growth phase. Once there is a shortage of nutrients or excess of waste products, the culture enters stationary and then decline phases. Bacteria respond to the starvation stress with growth

**Diagram 3. Bacterial Growth Curve**



rate reduction and induction of defense mechanisms [3, 4]. As a result, they may become more tolerant to biocides.

Cultures in exponential growth are actively metabolising and are also more susceptible to the effect of biocides due to increased free radical production from interference with normal metabolism. Bacteria usually become more resistant to environmental stress during slowing down of growth. This has been seen in both steady state and batch growth of cells and by comparing mid exponential with stationary phase growth large differences in efficacy have been demonstrated. The most tolerant cells are those in decline phase. While only viable cells will be counted to determine the initial cell concentration, the population may contain up to ten times the number of dead cells than viable cells. The dead cells will undergo lysis and release cell debris and intact enzyme systems that may interfere with the efficacy of the biocide.[3]




Test cultures may be prepared by growing them in a liquid medium or on a solid agar. Cells grown in liquid media have been shown to hardier and be less susceptible to antimicrobial attack than those washed from solid agar surfaces.

Almost all test methods include a requirement for the number of cells to be included in the inoculum along with how they are prepared. Cells grown in liquid medium or washed from solid agar require diluting to the required concentration. The dilution of the cells may result in a carryover of nutrients into the inoculum, particularly from liquid cultures. This may be removed by centrifuging and resuspending the cells in a non-nutrient solution, such as isotonic saline. Other test methods require the cells are diluted in nutrient broth [5] or even a 1:500 dilution of nutrient broth. [6] These variations result in different nutrient supply of necessary nutrients to the cells of to support growth and they may also inhibit the biocide under test.

The number of cells included in the inoculum is critical to the test result and will be further discussed later in this paper.

## Step 2. Add the organisms to the sample under test

The inoculum may vary in volume, placement and method of addition. The volume of inoculum added may affect the efficacy by diluting the test material if too much or repeated inoculations are conducted. The inoculum may be dripped in or onto a test samples, sprayed on and then mixed or left on the surface, all may affect the final outcome.

## Step 3. Allow a desired contact period.

The contact time of the microorganisms with the test material must be realistic and sensible. Disinfectant tests typically allow 8 minutes contact while preservative efficacy tests allow days and weeks to see an effect. These times reflect actual in use periods. Tests for antibacterial hand wash are typically conducted using 30 seconds and 5 minutes contact. Results for 5 minutes contact are often reported and are entirely unrealistic if the hand wash is to be rinsed off. However, if it is a leave on product, it may be a reasonable time. The standards for antibacterial surfaces and textiles utilizes a 24hour contact period [5, 7] which may be totally inappropriate, such as for nurses uniforms or antimicrobial cutting boards while acceptable for antibacterial socks that just need to stop growth rather than kill any organisms.

## Step 4. Neutralise the active

All antimicrobial tests require that the effect of the antimicrobial agent must be neutralized or stopped following the required contact time so that surviving organisms can be recovered. If this does not occur, the killing effect on cells may be allowed to continue well past the required contact time and may even inhibit growth of surviving organisms resulting in incorrect test results. If available a neutralizer specific for the antimicrobial is used, such as sodium thiosulphate for chlorinated water, or if not a general neutralizer, such as Lecithin and Tweens are used in varying concentrations. If it is not possible to neutralize the biocide then dilution of the test solution may be used. It is usual to find the result where no organisms are recovered reported as <10cfu/mL due to at least a 1:10 dilution in neutralizer. If this cannot be validated, then a result of <100cfu/mL is often seen indicating further dilution to 1:100 was necessary. It is usually regarded that if a 1:1000 dilution is required the validate the neutraliser system, then the test product is antimicrobial.

All antimicrobial tests require that the neutraliser is validated to ensure a correct result has been achieved.

## Step 5. Determine survivors

The enumeration method of surviving organisms may also affect the test result. The diluent and agar type chosen may influence recovery as surviving organisms may have been sub lethally injured. Agars with lower nutrient value, such as plate count agar (PCA), has been found to give better recovery than a high nutrient agar such as Tryptone Soya Agar (TSA). Counts conducted using the spread plate technique, spreading the inoculum over the surface of pre-poured agar plates, have been claimed to give a higher recovery than pour plates, where molten agar is poured onto the recovered organisms and mixed, due to less chance of heat stress on the surviving organisms. The counter argument is that pour plates give an additional dilution step thereby improving recovery of surviving organisms. Pour plates also have an increased sensitivity over spread plates due to a higher volume of inoculum into the plates.

## Step 6. Calculate Log reduction or % kill

Some methods require a log reduction, such as BP Preservative efficacy test, while others require % kill to be reported, as it usually seen for antimicrobial cleaners and disinfectants. The two methods use the same data but with different calculation methods. The log reduction compares the number of organisms surviving against the original number while the % kill compares the number of organisms killed against the original number added. The calculation methods are shown in table 2.

There is a direct correlation between log reduction and % kill as shown by the data in Table 3.

**Table 2. Calculation of Log Reduction and % Kill**

| Method | Calculation | Expressed as |
|---|---|---|
| **Log reduction (R)** | $R = \log_{10}(Ni \div Nx)$<br>$= \log_{10} Ni - \log_{10} Nx$ | Number,<br>e.g. 2 |
| **% kill** | % = (Ni– Nx)/Ni * 100<br>= (number killed/number added) * 100 | %,<br>e.g. 99.9% |

Ni: Number of microorganisms at time 0
Nx: Number of microorganisms at time x in the product

**Table 3. Comparison of Log reduction and % kill**

| Initial (Ni) | Recovered (Nx) | Killed (Ni – Nx) | Log reduction (log Ni-log Nx) | % kill [(Ni-Nx)/Ni]*100 |
|---|---|---|---|---|
| 5,000,000 | 500,000 | 4,500,000 | 1 | 90 |
| 5,000,000 | 50,000 | 4,950,000 | 2 | 99 |
| 5,000,000 | 5,000 | 4,995,000 | 3 | 99.9 |
| 5,000,000 | 500 | 4,999,500 | 4 | 99.99 |
| 5,000,000 | 50 | 4,999,950 | 5 | 99.999 |
| 5,000,000 | 10 | 4,999,990 | 5.6989 | 99.9998 |

**Table 4. Acceptance criteria**

| Test Method | Log reduction of surviving organisms | |
|---|---|---|
| | Bacteria | Moulds & Yeast |
| USP | ≥2 by 14 days and no increase thereafter | No increase in count throughout test |
| BP/EP option A | ≥2 by 2 days and<br>≥3 by 7 days<br>No increase thereafter | ≥2 after 14 days and<br>No increase thereafter |
| BP/EP option B | ≥3 by 14 days<br>No increase thereafter | ≥1 after 14 days and<br>No increase thereafter |
| CTFA (PCPC) | >3 by 7 days and no increase thereafter | ≥1 after 7 days and<br>No increase thereafter |
| CTPA | ≥3 by 2 days and no increase thereafter | ≥2 after 14 days and<br>No increase thereafter |

## Step 7. Report the results

Test methods generally require a number of factors be met to ensure the test is valid. These will include such things as the correct number of organisms in the challenge, the neutraliser was valid and the survival and recovery of the test organisms from untreated control samples.

If these are met a report can be prepared showing the calculated results. If the method contains pass criteria these can be compared to the results achieved and a statement of compliance with the test method can be made, such as "The sample tested meets Criteria A of the BP Efficacy of Antimicrobial Preservation". If, however, the test method contains no pass criteria the decision is to be made between interested parties, making a compliance statement difficult.

Laboratories accredited to ISO 17025 [ISO 17025 – 15 General Requirements for the competence of testing and calibration laboratories [8] are required to calculate uncertainty of measurements for all analysis conducted. This can be calculated but is not generally taken into account when reporting antimicrobial efficiency tests unless specifically requested. However, the 2017 version of the standard requires that measurement of uncertainty be taken into account when making compliance statements and only permits them if

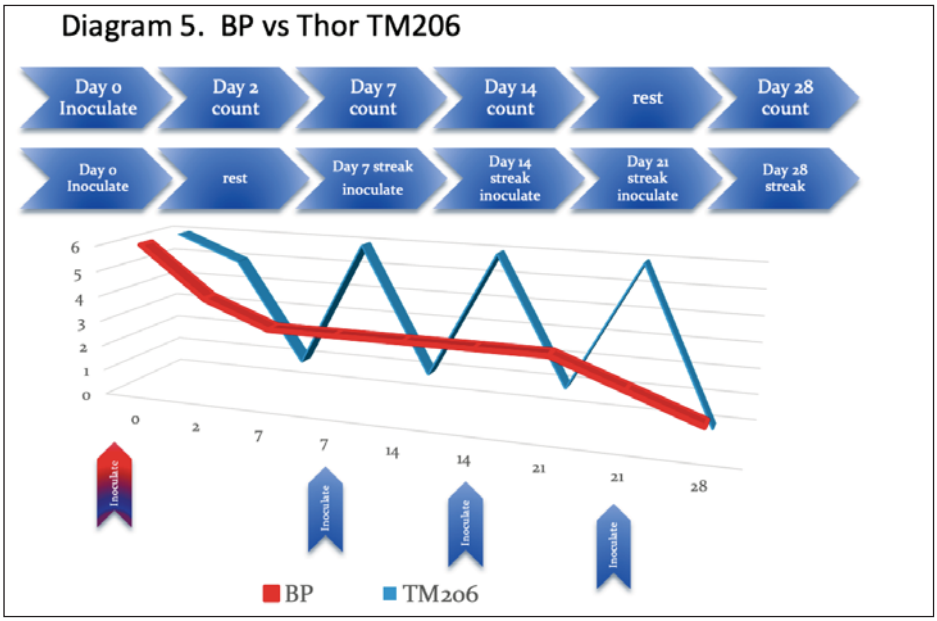
- the measurement results fall within the specification limits by an amount at least equivalent to the uncertainty of measurement; or
- the measurement results fall within the specification limits and the uncertainty of measurement is within the maximum permissible uncertainty prescribed in the specification; or
- the test specification defines the compliance decision rule to be used and the measurement results meet the specified criteria; or
- the customer and facility have agreed to a compliance decision rule.

Laboratories may not make a statement of conformity based on an agreement with the customer if the report is for the purpose of regulatory compliance. So, if the test report is part of the development or quality assurance process, a compliance statement may be made with no reference to measurement of uncertainty. But, if the report will be used to meet regulatory compliance, then either the uncertainty for each measurement must be calculated and used to determine if the results meet the requirements set out above, or no compliance statement may be included on the report.

## What do the test reports mean?

The best way is to look at a number of test procedures and see what the results actually mean.

Preservative Efficacy Tests (PET) may be conducted to a number of standards. They require that a range of defined micro-organisms are added to the sample under test and the sample is assayed at required times for surviving organisms. There are defined outcomes set for a reduction in the number of surviving organisms with time. The tests are designed to be reproducible and comparable and they gauge the




| Table 5 | | | | | |
|---|---|---|---|---|---|
| Test (inoculum concentration cfu/mL) | 2 days | 7 days | 14 days | 21 days | 28 days |
| BP Bact ($10^{5.7}$ - $10^{6.0}$) | 2 | 3 | 3 | 3 | 3 |
| USP Bact ($10^{5.7}$ - $10^{6.0}$) | - | - | 2±0.5 | 2±0.5 | 2±0.5 |
| TM206 Bact ($10^{6.3}$) | | 1.7 | 2 | 2.2 | 2.3 |
| TM206 Bact ($10^{6.9}$) | | 2.3 | 2.6 | 2.8 | 2.9 |

| Table 6 | | | |
|---|---|---|---|
| Test | Log Reduction | Reduction | Bacteria killed |
| BP | 2 log reduction at 2 days | 500,000 to 50,000 | 450,000 ($10^{5.65}$) |
| | 3 log reduction at 7 days | 500,000 to 5,000 | 495,000 ($10^{5.69}$) |
| TM206 low inoculation | 1.7 log reduction 7 days | 2,000,000 to 40,000 | 1,960,000 ($10^{6.29}$) |
| | 2 log reduction 14 days | 4,000,000 to 40,000 | 3,960,000 ($10^{6.59}$) |
| | 2.2 log reduction 21 days | 6,000,000 to 40,000 | 5,960,000 ($10^{6.78}$) |
| | 2.3 log reduction 28 days | 8,000,000 to 40,000 | 7,960,000 ($10^{6.9}$) |

effectiveness of the preservative system to control representative species. Their end points do not specify product sterility of the challenged product and they do not simulate in-use conditions or effects of packaging and as such only form part of the safety testing of a product. Clearly the test procedure meets the information given above. Pass criteria require minimum reductions in the number of surviving organisms calculated as log reductions. The required reductions for various Standard Test Methods are shown in Table 4.

Standard PET test tests require inoculating separate samples of the product under test once with individual organisms and measuring the number of surviving organisms at defined times. This method was developed for assessing preservatives in pharmaceuticals rather than cosmetics and differences in pack sizes, period after opening and use patterns lead to questions if the procedure is still the most applicable. The use of single organisms rather than mixtures is at odds with in use challenges where mixtures of organisms are more likely to be encountered and mixed organisms may assist each other in surviving or colonising a product. It is also unlikely that a product will only be challenged on one occasion soon after manufacture. The sources of microorganisms include the raw materials, production and filling equipment, packaging and the long term use by consumers. Loss of the preservative added to a product may occur quickly, due to incorrect pH or temperature exposure during production, or more slowly due to migration into the oil phase or micelles. Challenging the product several times may assist to show this problem.

A Thor test method TM206 was developed to overcome these shortcomings and relies on multiple inoculations of a mixed pool of test organisms.

The TM206 method sets a maximum recovery of surviving organisms rather than defining the number required to be killed, the log reduction, as required for most methods. Table 5 sets out the log reduction required by the BP and USP tests and this is independent of the number of organisms added, that is the number of organisms killed is not calculated, only that the number of survivors are 2 logs less than the number added. As the TM206 method sets a survival limit, the number added is significant. The data in table 5 shows the log reduction if the maximum or minimum number of organisms are added to the test. As additional organisms are added at each weekly challenge, the number required to be killed also increases as the number allowed to be recovered remains unchanged.

The requirements for log reduction indicate that the BP and USP tests are more severe as they require higher log reductions than the TM206 method. However, when the data is looked at to determine the number of organisms killed the results are the opposite. Data in table 6 shows the number of organisms killed when the lower inoculum concentration is used for Test TM206. The data clearly shows that despite a lower log reduction significantly more organisms are required to be killed to meet the test requirements.

The data from Table 6 highlights the issue with log reductions in that it shows the variation between the number of organisms added and the number surviving while giving no information on the numbers involved.

Tests conducted on antibacterial household cleaners or sanitisers often indicate their "strength" by promoting their germ-killing power with claims on their labels including the % kill. Diagram

Diagram 6. Product labels with Antimicrobial claims.


6 show label pictures of a disinfectant which kills 99.99% of germs along with a bathroom cleaning wipe and a washing detergent able to kill 99.9% of germs or odour causing bacteria. The test method for the disinfectants is mandated by the Therapeutic Goods Authority (TGA) under TGO 104 Standard for Disinfectants and Sanitary Products which recently replaced the long standing TGO 54 Standard for Disinfectants and Sterilants. The TGO 104 requires that all disinfectants have to meet the same standard to be labelled as disinfectants, depending on which class of disinfectant they are labelled as. However, antimicrobial cleaning products do not.

While there are limits on label claims they may make, no test methods are stipulated to determine how the 99.9% kill rates are achieved. Data presented in Table 7 comparing log reduction and % kill against the inoculum count clearly shows that a 3 log reduction, or 99.9% kill, can be achieved by killing anywhere from as few as 100 organisms to 9,990,000 organisms, all dependant on the initial inoculum count. Throw in using early exponential growth cultures, long contact times, poor inactivation, plates poured with hot agar, dodgy calculations and you can get almost any result you require.

Antibacterial textiles are also available either promoted to protect users from contamination or to extend the use of cloths between washing, particularly sports cloths. There are several methods for testing these claims, the most appropriate being the AATCC Test Method 100-2012 Antibacterial Finishes on Textile Materials, Assessment of. [5]

This method involves inoculating treated textile samples with *St. aureus* and *Klebsiella pneumoniae* and determining the number of surviving organisms after 24h contact. The log reduction is determined, but no pass requirement is set, it is up to interested parties to agree an acceptable result. The method requires that the test organisms are diluted in a nutrient broth when preparing the inoculum. An untreated control is also inoculated, and test organisms are required to show significant growth over the 24h test period.

The results show in Table 8 are for different sock types. The socks all include an antimicrobial thread at different concentrations and in different blends with other fabrics. The results for each sock type tested have implications for the effectiveness of the different blends trialled.

Table 7. Log reduction and % kill vs initial inoculum count

| Inoculum Count (cfu/mL) | 2 log reduction 99% kill | | 3 log reduction 99.9% kill | |
|---|---|---|---|---|
| | survived | killed | survived | killed |
| 10,000,000 ($10^7$) | 100,000 | 9,900,000 | 10,000 | 9,990,000 |
| 3,000,000 ($10^{6.48}$) | 30,000 | 2,970,000 | 3,000 | 2,997,000 |
| 1,000,000 ($10^6$) | 10,000 | 990,000 | 1,000 | 999,000 |
| 100,000 ($10^5$) | 1,000 | 99,000 | 100 | 99,900 |
| 10,000 ($10^4$) | 100 | 9,900 | 10 | 9,990 |
| 1,000 ($10^3$) | 10 | 990 | 1 | 999 |
| 100 ($10^2$) | 1 | 99 | 0 | 100 |

Table 8. Results of Tests on Antimicrobial socks to AATCC Method 100

| Sample | | *Staphylococcus aureus* ATCC 6538P | | *Klebsiella pneumoniae* ATCC 4352 | |
|---|---|---|---|---|---|
| | | *CFU/sample* | R value | *CFU/sample* | R value |
| Business sock | A 24hr | <100 | 3 | <100 | 3 |
| | B 0hr | $1.2 \times 10^5$ | | $1.2 \times 10^5$ | |
| Work sock | A 24hr | $6.4 \times 10^5$ | 0 | $4.1 \times 10^4$ | 0 |
| | B 0hr | $1.3 \times 10^5$ | | $6.1 \times 10^4$ | |
| Ankle sock | A 24hr | $1.3 \times 10^7$ | -2 | $1.9 \times 10^7$ | -2 |
| | B 0hr | $1.3 \times 10^5$ | | $1.1 \times 10^5$ | |
| Untreated Control | A 24hr | $1.2 \times 10^7$ | N/A | $8.2 \times 10^7$ | N/A |
| | C 0hr | $1.1 \times 10^5$ | | $1.0 \times 10^5$ | |
| Inoculum count | | $1.3 \times 10^5$ | | $1.1 \times 10^5$ | |

## What do they really mean?

The Preservative Effectiveness Test methods are generally consistent provided a standard method is used and if conducted correctly they will indicate if a sample under test has sufficient preservative available in the water phase to protect the finished product from microbial contamination. However, poor hygiene practices allowing growth of organisms in the process equipment and especially the development of biofilms, changes in the formulation or quality of raw materials or packaging may render the results invalid.

Incorrect maintenance of the test organisms, introducing variations to the inoculum preparation, including media type, growth phase and inoculum numbers may have a huge effect on the result achieved, failing a good

preservative system or passing a poor system.

As for antimicrobial claims on cleaning products and household items and textiles, the quality of the results is dependent on the test method used and the quality of the testing conducted. Interpretation of the results needs to be done with respect to the test method involved to ensure relevant organisms and contact periods have been used in the planning of the test and determining what reduction is really necessary to show that the product or article is either sufficiently preserved, that is, protected from microbial spoilage or that the antimicrobial product can actually meet the claims that the consumer expects.

The results for the socks tested shown in table 8 give very different results. All were for socks claimed to have antimicrobial effect allowing the socks to be reworn without washing. In use tests had shown the business sock could be worn for two months with no smell, the work sock for 1 month and the ankle sock for 1 week. The microbiological results were quite different from each other but mirrored the in-use tests for effectiveness. The results for the business sock would be sufficient to produce textiles blends able to self-sterilise, the work sock blend for textiles to self-protect and the ankle sock blend would not be recommended as an antimicrobial blend.

Results for antibacterial cleaning products may be tested to a recognised standard but if this is not revealed it is extremely difficult to tell what label claims actually mean. It is probably safe to assume that reputable companies are using reputable test methods, but this is not the case for all products on the market.

## Conclusion

There are many antimicrobial products available on the market including all products containing a preservative and those containing biocides that claim antimicrobial properties. There are many tests available to determine the efficacy of these. However, the particular test method utilised and variations introduced into these method by the testing lab may have a huge impact on the result obtained and therefore, the conclusions that can be drawn from those results.

An assessment of the method used, the initial inoculum concentration and contact times are the minimum variants that need to be included in determining if log reduction or % killed numbers quoted actually indicate a true antimicrobial effect for the product.

And, you always need to consider when you see a 99.9% kill: what happened to that 0.1% that got away and what is their significance?

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# A natural extract from sprouts to stimulate hair cycling and vitality

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## Introduction

Loss of hair strength, thickness, luster, density, and an unbalanced hair cycle are typical features of unhealthy hairs. Human Follicle Dermal Papilla Cells (HFDPC) are key to regulate the activity of the hair follicle.[1] Sprouts are considered functional food for their healthy value based on their rich composition in macronutrients, vitamins, flavonoids, phenolic acids.[2] Since herbal extracts have shown activity in HFDPC,[3] we tested sprout extracts as source of active molecules to stimulate HFDPC gene transcription. The in vitro study was then followed by a clinical trial that lasted four months and where several parameters associated with hair health were evaluated.

## Material and Methods

Seeds from Mung bean (INCI name: Vigna Radiata Sprout Extract) and from Red Clover (INCI name: Trifolium Pratense (Clover) Sprout Extract) were allowed to sprout in the laboratory. Extraction, followed by lyophilization provided a concentrated extract. HPLC analysis identified polyphenols and secondary metabolites.

The effect of the extract on HFDPC was evaluated after 24 and 72 hours incubation, followed by mRNA analysis using quantitative RT-qPCR for a panel of genes representing HFDPC physiology. Non treated cells were used as a control. Resulting data were statistically analyzed (Student's T test).

A double-blind, placebo controlled, clinical study was run on 26 men with alopecia. A water gel-based serum containing 1% of the extract was tested against a placebo. Measures of Anagen/Telogen ratio, hair quantity, hair density, scalp microcirculation were taken at the beginning of the study and after 28, 58, 84, 112 days. Pictures of panelists' hairs were taken as well. All data collected were statistically analyzed (Student's T test).

## Results

### In vitro Study (HFDPC)

| | |
|---|---|
| Insulin-Like Growth Factor 1 (IGF-1) | +142% |
| Bone Morphogenetic Protein 4 (BMP-4) | +67% |
| Structural Maintenance Chromosomes Protein 3 (SMC-3) | +48% |
| Platelet Derived Growth Factor-A (PDGF-A) | -52% |

Table 1. After 72h, the extract at 0.04% stimulated the transcription of signaling molecules associated with the maturation of the dermal papilla cells and with the activity of the hair follicle. Data were significant vs untreated.

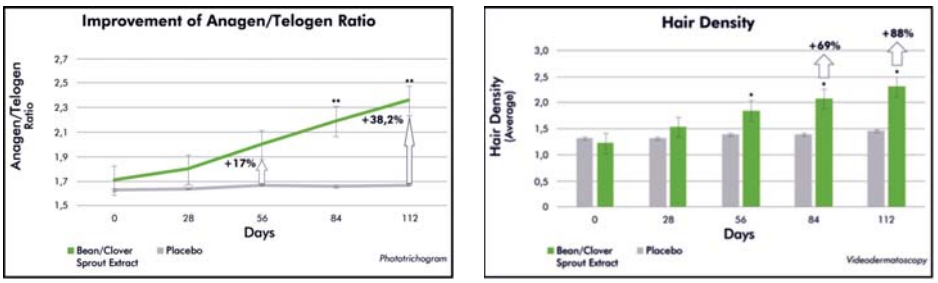


Figure 1. The serum containing 1% of the Sprout Extract increased Hair Anagen/Telogen ratio (Left). Hair density was also increased over time (Right). Asterisks indicate that data are statistically significant vs placebo ($p<0.01$)

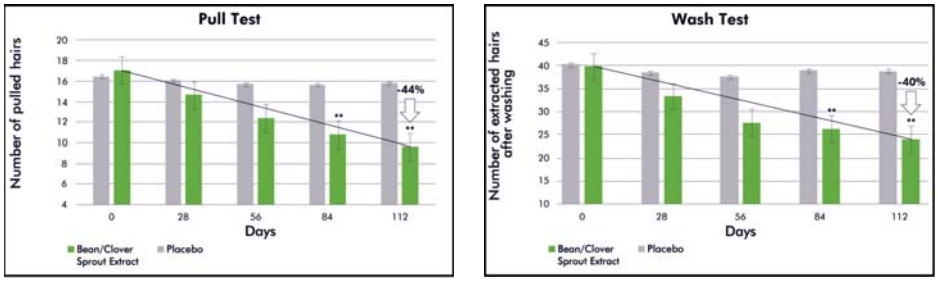


Figure 2. Hair Pull Test (Left) and Hair Wash Test (Right) show less hairs are lost over time when the serum containing the extract was used. Asterisks indicate that data are statistically significant vs placebo ($p<0.01$).

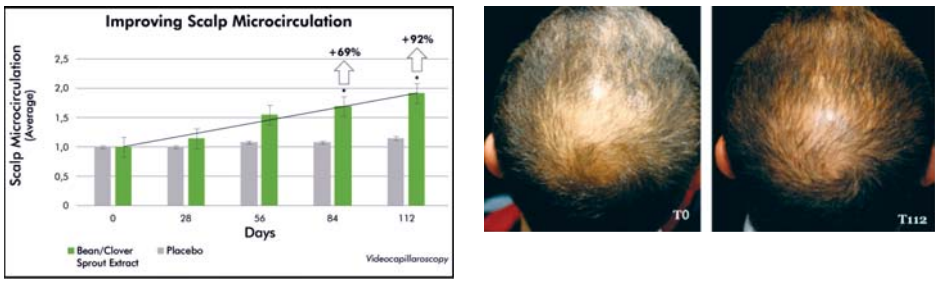


Figure 3. Left: Treatment with the serum containing the extract increased microcirculation over time. Asterisks indicate that data are statistically significant vs placebo ($p<0.01$). Right: A volunteer at the beginning (T0) and at the end of the study (T112). A visible increase of hair density is noticeable.

## Conclusion

A natural extract from sprouts was tested to improve hair quality and restore a vital hair cycle. in vitro data on HFDPC demonstrate stimulation of genes involved in growth, differentiation and signaling for a healthy and functioning hair follicle.[4-7] The clinical study shows that each parameter associated with a restored heathy hair is significantly improved in the group treated with the serum containing the extract but not with the placebo. Sprout extract (INCI: Trifolium Pratense (Clover)/Vigna Radiata Sprout Extract), can be suggested in hair treatment and conditioning products for stronger and healthier hair.

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In its long experience in the cosmetic filed Antonino worked in many areas for the cosmetic industry and developments, from the formulation to the industrialization of cosmetic finish products. In the last 10 year he was also involved actively and strongly in the R&D department of Akott Evolution.

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