

DNA BARCODING: PAVING THE WAY TO A NEW ERA OF SAFETY AND RELIABILITY

by Roberto Pace

Working with plants requires specific skills to handle their complexity and, consequently, to know exactly their metabolite patterns. The quality of herbal preparations relies strictly on the origins and quality of raw materials. Most plants can be unequivocally identified by a macroscopic and microscopic analysis but, in some cases, for a precise definition of the species or variety levels it is necessary to resort to DNA-based methodologies, which are proving to be powerful and highly reliable tools. DNA sequencing-based identification technologies complement the botanical, chemotaxonomic and metabolomic analytical methods and contribute decisively to the authentication of plants, but they are not the reference methods. It is of paramount importance to have "certified" samples of the species under investigation in order to derive reference DNA sequences for DNA method development and validation. As yet, the botanical extract industry does not have any such assurance. What really counts is gaining knowledge in plant genetic diversity and this knowledge is part of Indena's very own DNA. Our expertise in DNA barcoding puts together the whole array of DNA-based technologies, including DNA fingerprinting, PCR-based tools and specific DNA-Next Generation Sequencing.

Throughout our almost one-hundred-year history, we have been mapping the genetic identity of the raw materials used for our botanical extracts. We started integrating DNA sequencing into our Quality System in 2011, and have been putting together a precise database for starting material since then. Today, we possess a unique, priceless herbarium at the R&D site in Settala, which includes around five thousand classified and validated botanical samples that are not only used for macroscopic identification,

but are of pivotal support to the company's quality control department.

Recently, we developed a method dedicated to the genomic identification of botanical species based on unique sets of reagents for specific DNA sequences and the use of a miniaturized, state-of-the-art portable device for the analysis of nucleic acids. This device allows users, even unskilled personnel, to perform full genetic analysis of biological samples in any kind of setting. It incorporates all the necessary hardware, software and reagents and can be used as a fully portable testing laboratory to provide highly accurate results, reducing the time associated with traditional methods. DNA-based authentication of the ingredients incorporated into natural products is the most efficient and cost-effective, scalable method by which antagonistic effects of adulterants and plant or microbial contaminants may be minimized.

The topic of potentially mislabelled botanical products has recently raised questions about product quality and safety, leading to worries in general consumer confidence. DNA sequencing-based tests play an important role in those industries where adulteration is a serious concern, for example, with dietary supplements. At Indena we have always followed a rigorous scientific approach in our research and we have leveraged this together with our solid pharmaceutical background in the health-food market, where we develop biologically active ingredients for supplements, functional, medical and baby food products. In our long history, we have developed a huge number of products with a distinctive high quality profile, starting from a top level clinical study designed to meet and even exceed market expectations, turning challenges into opportunities. All the best products Indena brings to the market have undergone a standardization process and their activity in terms

of efficacy and safety is scientifically documented. We know that the use of every species of plant, and - sometimes - specific varieties within one species, may have substantially different significances on the results obtained at the end of the production process, in terms of secondary metabolites, the active ingredients.

Given this scenario, the need emerges to have a reliable genomic analysis, managed by experts who know and understand the plant kingdom and how to evaluate the best techniques in order to obtain the most useful information. Our approach to DNA barcoding is aimed not only at facing this challenge properly, but turning it into an opportunity. It is an approach which is completely reliable: we use all available techniques to develop methods and explore plant varieties, and the experienced botanists amongst our researchers are even able to identify species as yet unknown.

Indena is always committed to moving forward its market potential, to improving the high quality of the products and to seeking excellence in botanical extracts. At the same time, Indena acts proactively within the market, raising the bar in terms of expectations of quality, with the aim of making the whole industry a place where producers and consumers can trust and find products which are authentic and risk-free.

The pragmatism of Indena is paving the way to a new era of safety and reliability.



INDENA DNA TESTED

ENHANCING DEVELOPMENTS IN CUSTOM SYNTHESIS



In the coming weeks and exactly on schedule, Indena will complete the new GMP Kilolab for High Potency Active Pharmaceutical Ingredients (HPAPIs), a high containment facility for the clinical and commercial production of highly toxic active ingredients. The suite

is designed to handle the production of substances requiring an Occupational Exposure Limit between 20 and 50 ng/m³. This level of containment also allows for handling toxins, such as maytansine derivatives, which can be used by Antibody Drug Conjugates

(ADCs) manufacturers. Validation of the first API is planned for the first quarter of 2018; this will be a commercial API of total synthesis, whose production process was developed in recent months by the Indena R&D team. The new high containment Kilolab means that Indena will have the technical capacity to handle a greater range of compound classes, including derivatives from fermentation and total synthesis.

Although Indena remains firmly attached to its know-how of natural and semi-synthetic substances, the company will now be able to draw on its twenty years of high containment cytotoxic expertise to offer customers an extended range of custom synthesis. Analytical and research laboratories also form part of this new facility, so that all phases of the project, from development through to production and analysis can be managed in complete segregation.

MERIVA[®]: THE LIFE GUARDIAN[™]

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged or irritant cells. It is a protective response involving immune cells, blood vessels and molecular mediators. The function of inflammation is to inhibit the initial cause of cellular lesion, eliminate necrotic cells and tissues damaged by the original injury and initiate tissue repair.

It is a generic response and therefore is considered to be an innate immune mechanism, as opposed to adaptive immunity which is specific for each pathogen. Insufficient inflammation may lead to progressive destruction of the tissue by the harmful stimulus and the very survival of the organism may be at risk. On the other hand, chronic inflammation

can lead to a number of possibly serious pathologies.

Inflammation can therefore be classified as either acute or chronic. The former is the initial response to

the threshold of perceived pain, and consequently no immediate remedy is applied, exposing the body to a long and uncontrolled damage.⁽¹⁾

In addition, chronic low intensity inflammation is part of our physiological aging process.⁽²⁾

In Western culture alas, we are used to turning to pharmacology only when confronted with evident symptoms. It is an approach that is slowly changing, as more and more people consciously follow healthier lifestyles. Clearly, keeping

inflammation levels under control is a far-sighted way not only prolonging life but prolonging a better life. *Life-long maintenance* is a personal life project.

We can help our bodies keep inflammation levels under control, perhaps with a small daily gesture such

a harmful stimulus, which may be physical, chemical, or derived from biological agents; typical symptoms are reddening, swelling, pain and loss of functionality. The latter, known as a "silent killer", is more prolonged since it remains below

as supplementing our diet with a proper dose of curcuminoids. Meriva[®], the standardized turmeric extract in the form of Phytosome[®], helps the body keep this *silent killer* under control acting as a real "Life Guardian™" in protecting the well-being of the body. And it can do so the whole life long. This is because Meriva[®] combines three basic elements: its anti-inflammatory effectiveness, typical of curcuminoids contained in turmeric, its long-term tolerability and specific clinical efficacy data.

In order to optimize absorption in humans of the curcuminoids, which normally offer poor bio-availability, Indena has opted for a biomimetic approach, using Phytosome[®] technology which has a number of key features: it contains only food grade excipients, phospholipids, endemic to our organism

and no other pharmacological remedy, which make Meriva[®] ideal even for use over long periods.

It does not aim at maximum performance in terms of bioavailability, but to optimize the amount of active substances, avoiding the risk of unnecessary formula overdosing, in accordance with the principles of *food-equivalence* and Nature as Measure™.

Meriva[®] is standardized to contain all three curcuminoids in turmeric, respecting the same proportions that nature has developed over millions of years of evolution. Each unit contains 200 mg of curcuminoids - the average daily intake in an Indian diet. The use of phospholipids mimics an Indian food tradition that is to mix turmeric in milk for the preparation of what they call *golden milk*.

Having therefore taken these principles as benchmarks in the design and development of this ingredient, the efficacy of Meriva[®] has now been validated by the results of 29 clinical trials in ten different health conditions. So all you need is 1g of Meriva[®] a day, along with a healthy lifestyle, to provide a valuable aid in protecting the body from silent inflammation.

MERIVA[®]: THE LIFE GUARDIAN™.

[1] Anne M. Miniñane, Low-grade inflammation, diet composition and health: current research evidence and its translation. *British Journal of Nutrition* (2015), 114, 999–1012.

[2] Calçada D, The role of low-grade inflammation and metabolic flexibility in aging and nutritional modulation thereof: a systems biology approach. *Mech Ageing Dev.* 2014 Mar-Apr;136-137:138-47. doi: 10.1016/j.mad.2014.01.004. Epub 2014 Jan 23.

PROTECTING THE SKIN AGAINST POLLUTION

Our skin is physiologically equipped to defend us from the harmful effects of atmospheric agents; nevertheless, with the advent of industrialization and the spread of urban sprawl (it is estimated that by 2050, 66% of the world's population will live in highly urbanized areas), environmental and especially airborne pollutants threaten the intrinsic ability of our skin to protect us.

There is however a new natural ingredient, recently validated both *in vitro* and *in vivo*, that has proven to be effective in

preventing skin damage from air pollutants. Cultures of fibroblasts exposed *in vitro* to damage from heavy metals benefited from a marked and significant effect in protecting cell vitality by a new active ingredient named Vitachelox™, a ternary mixture of natural extracts rich in polyphenols. Validation on the various cellular segments showed its more specific protective capacity, respectively with the different cellular components: lipids, proteins, and DNA.

Thirty volunteers, half of whom in Europe and half in China, were exposed for six consecutive hours to polluted air as part of their daily routine; subsequent topical clinical assessment highlighted the effective protection capability of Vitachelox™ on the skin. The active substance, compared to the placebo, was found using atomic absorption tests to completely prevent the deposition of heavy metals within the *stratum corneum*, thereby averting any damage to the cells.

Heavy metals, although

present in small quantities among air pollutants, are in fact among the most harmful compounds since they can catalyse harmful oxidative reactions, although not stoichiometrically. In other words, they trigger the reaction without being directly involved and are not therefore consumed. Thus, the evidence that Vitachelox™ can prevent heavy metal deposits, albeit in quantities of the order of ppb or ppm for iron, is a very accurate indicator of the ability to prevent the harmful effects of pollutants on skin, such as accelerated aging, hyperpigmentation and a greater likelihood of inflammatory conditions such as atopic dermatitis.

To show how the product can be used in practice, two formulas *Daily Defense* and *Energy Enhancer* were created in line with the modern trend for multifunctional products and were presented at the recent In-Cosmetics show. Each one contains several active ingredients which in synergy with protection from Vitachelox™ complete and enhance the effectiveness of those multi-purpose coloured creams which are half protective and half make-up and very much in demand today.



CASPEROME®: FRESH EVIDENCE OF IBS RELIEF

Irritable Bowel Syndrome (IBS) is a chronic, relapsing, gastrointestinal disorder in which abdominal pain or discomfort is associated with change in bowel habits. It affects 10-20% of the adult population worldwide and is often unrecognized or untreated. Considering the chronic nature of this disorder, IBS can have a strong negative impact on the quality of life of those who suffer from it.

A first clinical study demonstrated that the Indena lecithin-based delivery form of *Boswellia serrata* extract (Casperome®) attenuated symptoms associated with mild ulcerative colitis in remission,^[1] as the Phytosome® formulation remarkably increased its bioavailability and penetration across biological membranes.^[2,3] These two features strengthen the potential effectiveness of Casperome® in

needed or supplementation with Casperome®, 1 tablet of 250 mg per day. The observational period was 4 weeks and conventional drugs were considered as rescue medications should Casperome® supplementation fail to alleviate IBS symptoms. All IBS symptoms investigated improved during the period with the three management strategies, but only in the Casperome®-supplemented group did the number of

a mechanism of action that involves the modulation of the natural inflammatory response. Casperome® is the only formulation of *Boswellia serrata* extract available on the market which is supported



by clinical outcomes validating its efficacy profile also in the management of gastrointestinal disorders such as IBS.

The Phytosome® technology confirms its key role in the bioavailability of active principles: triterpenoid acids of *Boswellia serrata* are poorly bioavailable, Casperome® is able to optimize their absorption and to deliver their full natural bouquet, thus rapidly alleviating the symptoms of IBS.

subjects with any IBS symptoms significantly decrease, from 58% to 12.5%. In addition,

the prevalence of side effects was significantly higher in the two groups treated with the Standard Management Strategies (28% for group 1 and 26% for group 2) compared to Casperome® supplemented subjects (8.2%). The extracts of *Boswellia serrata* were demonstrated to be effective in the management of various inflammatory response functions including those that occur in the bowel, joints, bones, respiratory airways and in the brain, with

IBS which Indena sought to investigate further with an additional clinical study.

The study enrolled 71 otherwise healthy subjects with idiopathic IBS and divided them into three groups according to different management strategies: hyoscine butylbromide, administered when needed, papaverine hydrochloride 10 mg + belladonna extract 10 mg, administered when

[1] Pellegrini L. et al., Managing ulcerative colitis in remission phase: usefulness of Casperome®, an innovative lecithin-based delivery system of *Boswellia serrata* extract. *Eur Rev Med Pharmacol Sci* 2016;20:2695-2700.

[2] Hüsich J. et al., Enhanced absorption of boswellic acids by a lecithin delivery form (Phytosome®) of *Boswellia serrata* extract. *Fitoterapia* 2013;84:89-98.

[3] Riva A. et al., A single-dose, randomized, cross-over, two-way, open-label study for comparing the absorption of boswellic acids and its lecithin formulation. *Phytomedicine* 2016;23:1375-1382.

[4] Belcaro G. et al., Supplementation with a lecithin-based delivery form of *Boswellia serrata* extract (Casperome®) controls symptoms of mild irritable bowel syndrome. *P Eur Rev Med Pharmacol Sci* 2017;21:2249-2254.

EMPLOYEE HEALTH AND SAFETY FIRST

Indena is proud to have achieved OHSAS 18001:2007 certification for the Settala and Tours production facilities in Italy and France. This certification is recognition of Indena's policy and management procedures implemented for employee health and safety in the work place.

The company is strongly committed to preventative and protective measures as well as continuous improvement of working conditions in every area of the production process. The certification is part of the internationally recognized Occupational Health and Safety Assessment Series which is designed

to guarantee that adequate policies are in place and managed systematically, continuously and efficiently.

The OHSAS 18001:2007 complements the ISO 14001 certification related to the Environmental Management System, which Indena had already obtained for both its main production sites in Settala and Tours, in a bid to maintain its leading position as a producer of active principles derived from plants. The next goal will be to obtain certification for the Group's other industrial sites in Europe.



PARTNERSHIPS FOR THE FUTURE

The commitment of Indena to innovation and development has for many years linked the company to academic and industrial partners both in Italy and internationally. An important part of this involves obtaining Institutional funding for research to validate and support projects that the company has undertaken. Indena is playing a significant role in two such projects GRACE and COGNIPANT, which were set up recently and appear particularly interesting and promising.

GRACE which stands for GRowing Advanced industrial Crops on marginal lands for bioRefineries is a European project, linked to the Horizon 2020 Framework, developed by a consortium of 22 companies and universities. It is funded by the Biobased Industries Joint Undertaking (BBI JU), a public-private partnership between the European Union and the Bio-based Industries Consortium (BIC), and aims to sustain and develop bio-economic alternatives to fossil fuels. The project began in June 2017 and will last until May 2022. It envisages specific land reclamation initiatives and the enhancement of marginal areas through the cultivation of plants such as *Miscanthus* and industrial hemp which offer potential added value. A company called Ecohemp together with the Università Cattolica del Sacro Cuore and the Consorzio di Bonifica di Piacenza will grow industrial hemp. The intention is to reclaim the land

for cultivation and obtain hemp fibre and oil; Indena will then recover the chaff remaining after harvesting to isolate non-psychoactive cannabinoids (CBD, CBDV, CBG), destined for pharmaceutical and cosmetic use or as an intermediate for semi-synthesis. The initiative is a further step towards the concept of circular economy.

The second, COGNIPANT was launched by Indena in collaboration with the Università degli Studi di Milano. It is an industrial research and experimental development project funded by the Regione Lombardia, D.G. Università, ricerca e open innovation, with the participation of two partners of excellence: Axxam S.p.A., the international leader in the development of *in vitro* models and Gricar Chemicals S.r.l., a leading manufacturer of finished product formulations. The project centres on the study of new botanical derivatives aimed at the prevention of cognitive deficits in adulthood and aging, from the definition of a biomedical profile and formulation to the best delivery system for the finished product. Cognitive decline is a field in which there is still a great deal of research to be done, as there is currently no pharmacological therapy to administer effectively alongside cognitive-behavioural therapies. The eighteen-month project aims to explore new research and development opportunities, with a view to improving quality of life and addressing medical issues as yet unresolved.

THE BRAZILIAN SOUL OF MERIVA®

Master Meeting, an event dedicated to the world of pharmaceuticals is into its third edition. This year the meeting, organized by Aché and held in the Brazilian resort Arrial d'Ajuda - Bahia, focused on osteoarticular therapies for which Aché markets two products: Motore® (Meriva®) and Artrolive® (Chondroitin + Glucosamine).

Issues relating to these therapies, with particular reference to the management of pain and inflammation, were explored during the conference.

Paolo Morazzoni illustrated the properties of *Curcuma longa*, and its unique clinical benefits when administered in the form of Phytosome® as Meriva®. Since the launch of Motore®, the scientific evidence in support of Meriva® has continued to grow, validating this active ingredient in over ten different health conditions.

The audience of around ninety medics, mostly rheumatologists, showed their appreciation for the talks and participated fully in roundtable discussions where their many requests for further information were given detailed answers by the respected experts.

SUSTAINABILITY: A POINT OF NO RETURN FOR INDENA

Indena focuses on social and environmental sustainability with ever increasing commitment: in 2017 our production sites both in Italy (Settala) and in France (Tours) obtained OHSAS 18001 certification (Occupational Health and Safety Assessment Series); an internal procedure for compliance with the Nagoya Protocol came into force in 2015; projects in co-operation with NGOs are ongoing and we make constant efforts to ensure a sustainable supply of botanical raw materials; presentations of our policies are made in trade shows and as part of higher degree courses.

The Sustainable Sourcing Programme (SuSo) started in 2013 with the creation of a multidisciplinary team and a careful analysis of supply chains taking into consideration the protection of communities involved in plant cultivation, harvesting and processing, respect for biodiversity and environmental protection. The results obtained led to the launch of projects which are still underway in several parts of the world.

The first project was undertaken in the Alaotra Mangoro region of Madagascar, where *Centella asiatica* is grown. Wells are being sunk, and renovation is taking place in five elementary schools where support began in 2015. 1,400 school kits have been given to students, along with teaching material and teacher training courses in methodology, pedagogy and French.

Improvement in school literacy rates and examination results confirm the success of the project which was developed and coordinated with the help of our local supplier and Reggio Terzo Mondo, an Italian NGO which has worked in Madagascar since 1973. The initiative won the CPhI Pharma Award "Excellence in Pharma: Corporate Social Responsibility" in October 2016.

A second project launched in 2016 and dedicated to the two village communities in the Madhya Pradesh region of India where the resin used to produce Casperome® and Bosexil® is obtained from the *Boswellia serrata* tree. With



the support of our supplier and a local mission, *Gwalior Catholic Seva Samaj*, warehouses have been built for the preservation of raw materials, leading to an improvement both in working conditions and in material quality. The supply chain has been simplified and a community space created to enable storage of other goods as well as training provided on sustainable harvesting.

Another interesting project is unfolding in three villages in the Dodoma, Tanga and Kibaha areas of Tanzania. Here associations have been formed to ensure the conservation and sustainable management of Miombo, a natural forest formation at risk of disappearing, due to the pressure of agricultural expansion and the population's growing need for fuel. The Miombo forest is an ecosystem and home to *Terminalia sericea*, a tall tree from which Indena obtains Sericoside, a useful cosmetic ingredient for anti-aging applications. The project was set up with the Centro per un Appropriato Sviluppo Tecnologico (CAST) a charity founded in 1980 by people with voluntary service experience in Africa with the aim of cooperating with third-world countries to foster self-development. The project is also supported by the Tanzania Forestry Research Institute (TAFORI), the Tanzanian Forest Service (TFS), the local supplier and Luigi & Felice Castelli, the exclusive distributor of Indena cosmetics in Italy.

Also in this case the success of the project is due to the company, supplier, local

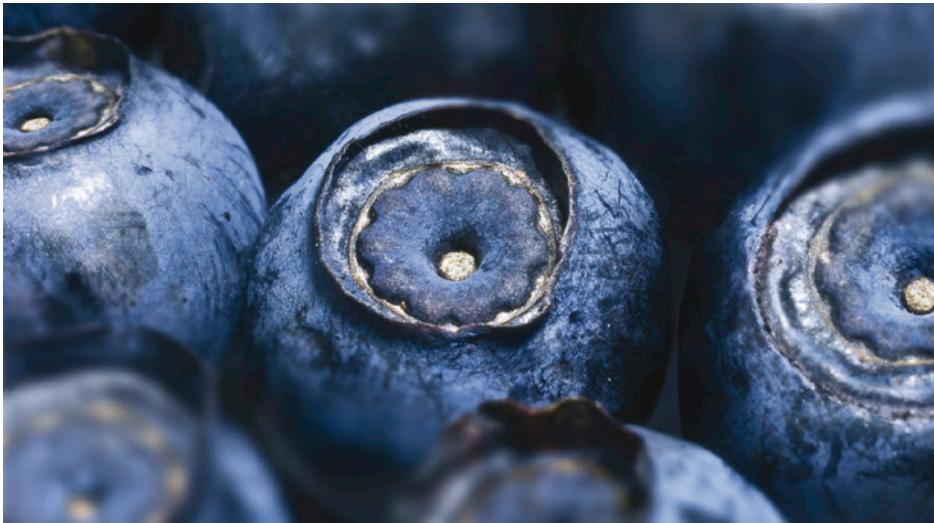
charities institutions and the community all working together observing and responding to real needs. Nationally-recognized associations able to interact on forestry with the technical institutions have been assigned land by the state to use as a "model forest" for learning and applying sustainable practices. Practical and theoretical training sessions have already been held in the three areas on forest management, the cultivation and conservation of *Terminalia sericea* and its integration into the agricultural system. The plan also includes setting up beekeeping and selling honey which will generate an important source of income, particularly for women. Work on the ground, training and co-operation with local authorities are the key points in a project that for the first time promotes a sustainable approach to forest management in the Tanzanian savannah. This is seen as an important innovation as well as an earning opportunity in keeping with protecting the environment.

Indena continues to implement projects and initiatives in order to work ever more systematically and thoroughly on its supply chains. For this reason, the company has joined Sedex, a British non-profit organization whose aim is to foster worldwide the sustainable management of supply chains, utilizing one of the largest data collection platforms in Europe to monitor the ethical behaviour of companies.

THE BEST BILBERRY EXTRACT AVAILABLE, SINCE 1970

We have been working on bilberry extract since the early seventies. Over the years, our interest in the stunning properties of the precious small bluish berry and our standardized extract Mirtoselect[®] has been unique: today it is not only the

disturbance. The new randomized, double-blinded, placebo-controlled clinical study was published in the European Review for Medical and Pharmacological Sciences^[1] and it offers further proof of the efficacy



most extensively studied bilberry extract available, but the market benchmark. Thanks to its antioxidant potential, the major applications investigated so far for Mirtoselect[®] are in the realms of vascular health and ophthalmology, but animal models and pilot clinical studies may suggest a broader clinical profile of anthocyanins, that encompasses memory improvement, cardiovascular health, metabolic syndrome and obesity. New positive data have recently emerged regarding the supportive role of Mirtoselect[®] in eye health, in particular dry eye conditions, where preserving adequate tear levels and maintaining the ocular surface healthy may possibly help in attenuating discomfort and visual

and safety profile of Mirtoselect[®], already supported by over 30 clinical studies. Indena is indeed proud of the fact that today Mirtoselect[®] is the only extract of *Vaccinium myrtillus* supported by a clinical study on this specific eye dryness condition which affects millions of people the world over. Indena brings to the market, to customers and to the final consumer a level of quality that few others are able to guarantee; one of the finest examples of this quality is Mirtoselect[®].

[1] Riva A. et al., The effect of a natural, standardized bilberry extract (Mirtoselect[®]) in dry eye: a randomized, double blinded, placebo controlled trial, European Review for Medical and Pharmacological Sciences, 2017; 21: 2518-2525.

INDENA EVENTS CALENDAR

CPH Worldwide 2017

Frankfurt, Germany
24-26 October 2017
Messe Frankfurt
Stand 8.0F30

Food Ingredients Europe 2017

Frankfurt, Germany
28-30 November 2017
Messe Frankfurt
Stand 09.0B24

In-Cosmetics 2018

Amsterdam, Netherlands
17-19 April 2018
RAI Amsterdam
Stand M138

Food Ingredients China 2018

Shanghai, China
22-24 March 2018
National Exhibition
and Convention Center (Shanghai)
Stand 61H05

Vitafoods 2018

Geneva, Switzerland
15-17 May 2018
Palexpo
Stand H34

For the complete
events list, visit
indena.com

CPH China 2018

Shanghai, China
20-22 June 2018
SNIEC Shanghai
Stand E1A17

Vitafoods Asia 2018

Singapore
11-12 September 2018
Sands Expo & Convention Centre
at Marina Bay Sands



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