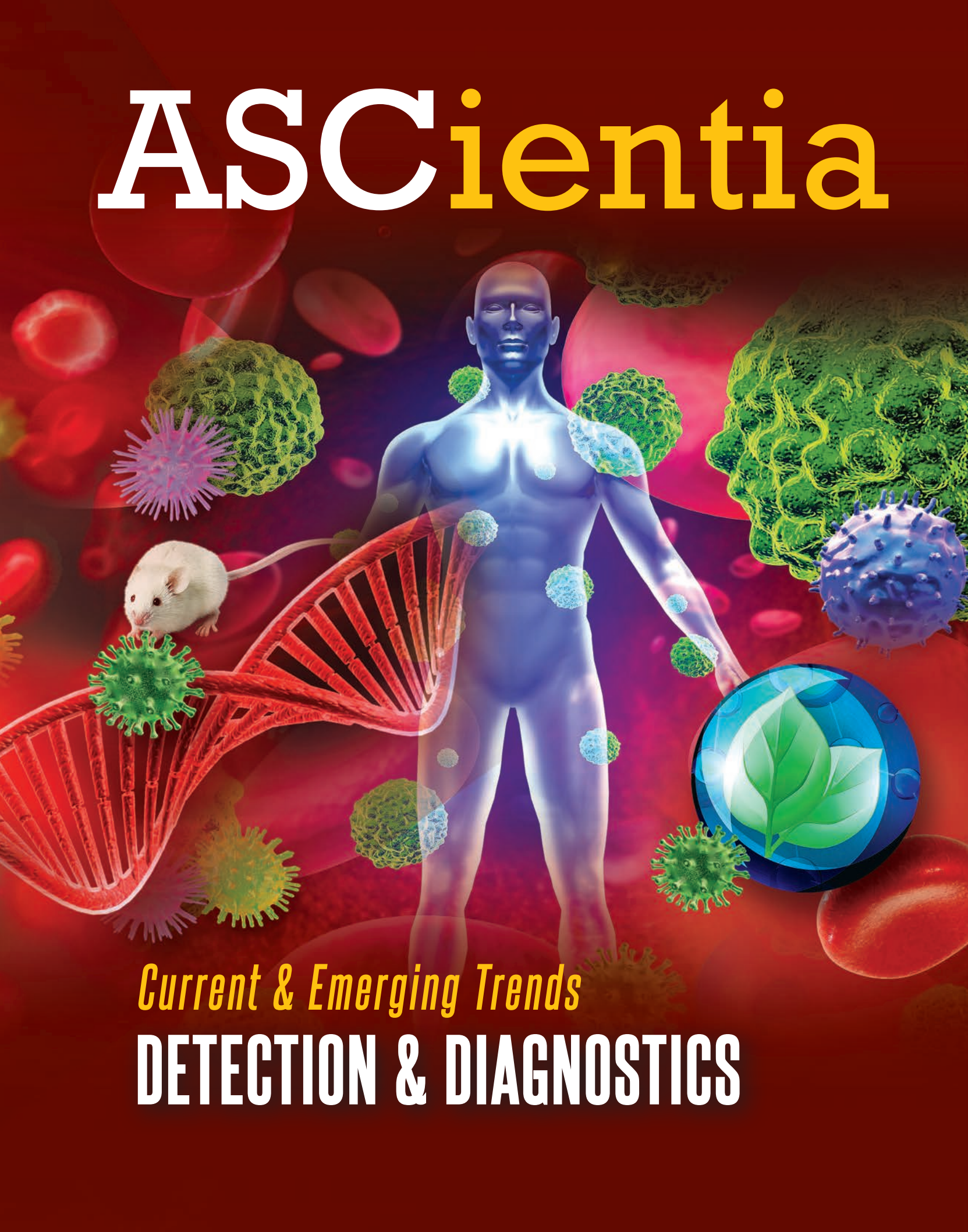
ASCientia



Current & Emerging Trends

DETECTION & DIAGNOSTICS

Contents

Issue No. 12 (2017)

ASCientia is a combination of ***ASC*** (acronym for School of Applied Science) and ***scientia*** (Latin word for knowledge, science and skill)

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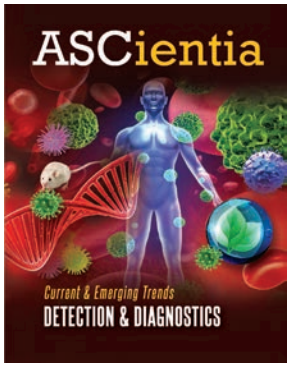
School of Applied Science



DIRECTOR'S MESSAGE

This year is going to be momentous with many exciting developments. School of Applied Science is proud to host **ASConference** 2017 portraying the technological advancements in different industry sectors and achievements of our domains. We have well established diagnostic capability serving the various stakeholders in analytical and bioanalytical testing which is well aligned with the conference theme of *Current & Emerging Trends: Detection & Diagnostics*.

The pace and promise of scientific research and technology development are tremendously exciting for diagnostics which has played an increasingly significant role in the early recognition, diagnosis and management of diseases. Today, we stand on the threshold of another explosion in the development of diagnostic technology that will cater to more targeted therapies together with complementary diagnostic tests. We are discerning the convergence of technology with digital health.

In precision medicine, our knowledge of the genomic aspects of health conditions is growing steadily. Molecular diagnostics are quickly becoming essential tools at every stage of the healthcare field, from discovery to R&D; to diagnosis and prognosis; to treatment selection, and to effective patient monitoring.

Informatics is another area of diagnostics that is gaining momentum as diagnostic companies move beyond generating test results. This will have applications everywhere from biopharmaceutical discovery to remote patient monitoring and population health management, ranging all the way from the clinic or individual hospital to national public health applications.

Our Centres of Excellence (COEs) and Centre of Innovation for Complementary Health Products (COI-CHP) have core lab capabilities that can provide quality and cost-effective testing services. When speed of response is important, or where on-site analysis or monitoring is needed, point-of-care testing platforms will be engaged with. Our on-going research focuses on integrating core diagnostic capabilities with broader informatics and digital heath functions to revolutionise diagnostics technology in healthcare and medicine.

"Better Tests: Better Care: Improved Diagnostics for Infectious and Non-Infectious Diseases."

We have the diagnostic capability to collaborate with you. We invite you to join our long list of industry collaborators who have already benefitted from our consultancy projects.

Partner us, make your business better.

Lee Chee Wee, PhD

Rapid On-site Detection of Fish Iridoviral Disease

Padmanabhan Saravanan, PhD
Head/Centre of Innovation for Complementary Health Products

Intensification of fish aquaculture in sea-based and land-based farms to beef up the productivity, and the movement of large volume of ornamental fishes; around 1.5 billion fishes comprising ~2000 different species, exposes the fish industries to a rapid transmission of diseases. Iridoviruses (Figure 1) causes systemic diseases in fishes. They are a significant cause of mortality in more than 32 other species of cultured marine and freshwater fishes and a few species of ornamental fishes such as the Gourami species, Dwarf gourami, angel fish, guppies, platys and doctor fish.

There is a need for on-site iridoviral disease diagnostics that could potentially help in farm biosecurity risk management. The aquaculture team in Temasek Polytechnic has developed an optical immunoassay (OIA) based iridovirus detection kit "Irido-OIA" that acts as a screening tool for on-site sample processing and detection of iridoviruses in fishes.

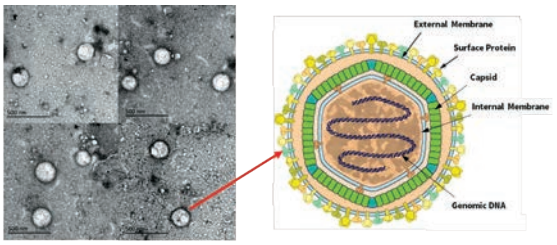


Figure 1: *Electron micrograph of iridovirus isolated from sick seabass fish (left) and schematic on the structure of the virion (right)*

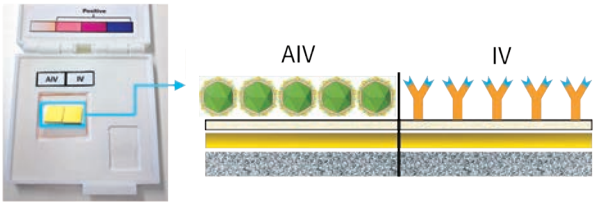


Figure 2: *Optical immunoassay (OIA) device for iridovirus (left) that can detect both virus (IV) and host response (AIV) simultaneously. OIA chip test schema as shown (right)*

The kit comprises sample processing (tissue and blood) gadgets, and a disposable test device that can detect both virus and host response (specific antibody response) in the same device (Figure 2). The assay works on the principle of thin film biosensor. Changes in optical thickness on chip surface due to immunobinding events alters the path of reflected light causing a distinct visible colour change from gold to purple under white light. Figure 3 depicts the user-friendly on-site sample collection, processing and detection steps that enable farm hands to do testing in less than 30 minutes. The detection sensitivity was determined to be 5x103 viral particles per millilitre of processed tissue sample. Operational advantages of the Irido-OIA kit includes it being field deployable, quick to detect diseases and its user-friendliness with less logistic load and visual read-out.

Figure 3: *Sample preparation on-site for iridovirus detection. From left to right showing tissue collection, virus concentration, testing on OIA cassette, positive result showing purple blue colour development*

The developed kit could have potential advantages to the aquaculture industry in routine animal health monitoring, quarantine management, vaccine response analysis and the screening of sick fish for appropriate containment measures.

Figure 4: *Iridovirus-OIA kit with sample extraction gadgets and test device*

Rapid On-site Detection of Food Contaminants

Nurshahidah Ali, PhD
Research Fellow/Biological Testing

Alden Toh
Project Officer/Biological Testing

The increasing worldwide trend of foodborne outbreaks is a cause for concern. A lacunae exist in current testing methods in terms of 'fieldability', long analysis time and cumbersome procedures. There is a need for rapid on-site diagnostic capability to shorten screening time which will help mitigate the impact of an outbreak.

The project team has successfully developed a novel fieldable method based on Optical Immunoassay (OIA) that can concentrate and detect biological contaminants in food with a total analysis time of less than two hours. This innovative detection kit uses customised extraction tools and can detect multiple pathogens/toxins in a single test at reduced cost. Validated by DMERI, DSO National Laboratories and analysed by the Environmental Health Institute, NEA for user-friendliness and overall performance, this kit has been used in student projects to detect Staphylococcus bacteria in food samples from school canteens.

The kit is expected to enhance foodborne illnesses-related investigation for timely risk mitigation. It can also be tailored to detect a wide range of pathogens, toxins and DNA.

Figure 1:
Components of the OIA biotoxin detection kit

Particle Filtration Efficiency of Endonasal Devices

Nurshahidah Ali, PhD
Research Fellow/Biological Testing

Alden Toh
Project Officer/Biological Testing

Padmanabhan Saravanan, PhD
Head/Centre of Innovation for Complementary Health Products

Exposure to pollution from air particulates has been related to a variety of health issues in humans, especially respiratory allergies. Singapore's National Environment Agency (NEA) uses standards set by the United States Environmental Protection Agency (USEPA) to gauge air quality. The pollutant standards index basically comprises of six pollutants viz. sulphur dioxide (SO2), particulate matter (called "respirable suspended particles (RSP)" or PM10, as they are 10 microns or smaller in size) and fine particulate matter (PM2.5), nitrogen dioxide (NO2), carbon monoxide (CO) and ozone (O3). PSI levels exceeding 300 is considered hazardous. The USEPA standard for 24-hour mean PM10 level and PM2.5 level is 150 µg/m3 and 15 µg/m3 respectively. Unhealthy range of PSI 200 and 300 values for RSP are 374.43 and 625 µg/m3 respectively.

United BMEC has come up with a novel solution to overcome this problem by using endonasal devices PPE and City & Travel (Figure 1). PPE-type and City & Travel endonasal devices falls under Cat III and Class I Medical Device according to the European Directives 89/686 and 93/42 respectively.

ASC has built customised testing capability for endonasal devices (Figure 2) to assess the performance of particle filtration efficiency of these devices. The study was conducted using simulation of hazardous PSI conditions with silicon dioxide particles representing PM10 and PM2.5 range and an artificial lung breather.

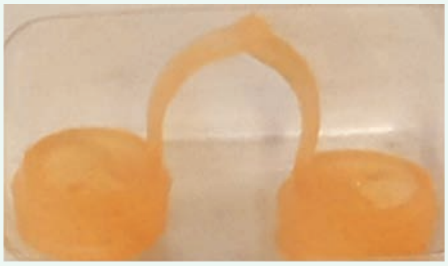


Figure 1: *Endonasal Device*

The devices demonstrated a particle filtration efficiency of 90% at high relative humidity (RH) for a PM10 range over an 8-hour period and 4-5 hours for the fine particulate range (PM 2.5). The efficiency dropped to 75% at the 8-hour time point. Difference in the fine particle filtration efficiency was observed with fine particulate range (PM2.5) at low RH.

The endonasal device PPE may perform efficiently for up to 8 hours under normal - unhealthy levels of PSI, and for up to 4 hours under a hazardous range of PSI subject to breathing resistance. The City & Travel device may perform efficiently for up to 6 hours under normal - unhealthy levels of PSI and for 2-4 hours under a hazardous range of PSI.

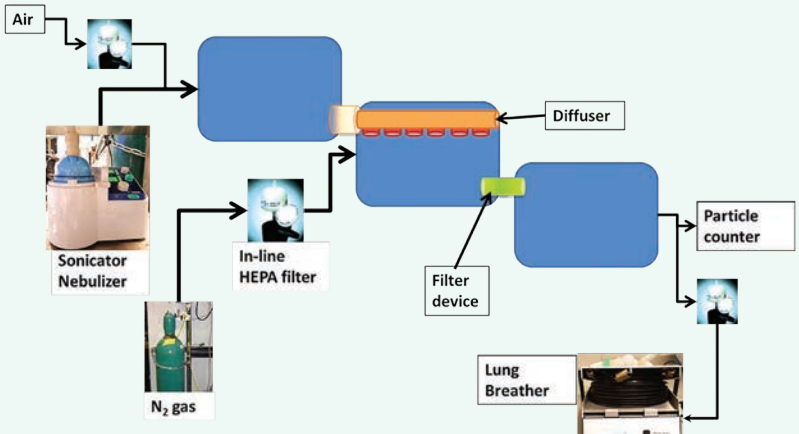


Figure 2: *Schematic of the endonasal device test set-up*

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	White rice long grain (Jasmine)	GI 78
	Brown rice (short grain)	GI 76
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	Basmati white rice	GI 64
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Source: Glycemic Index Research Unit

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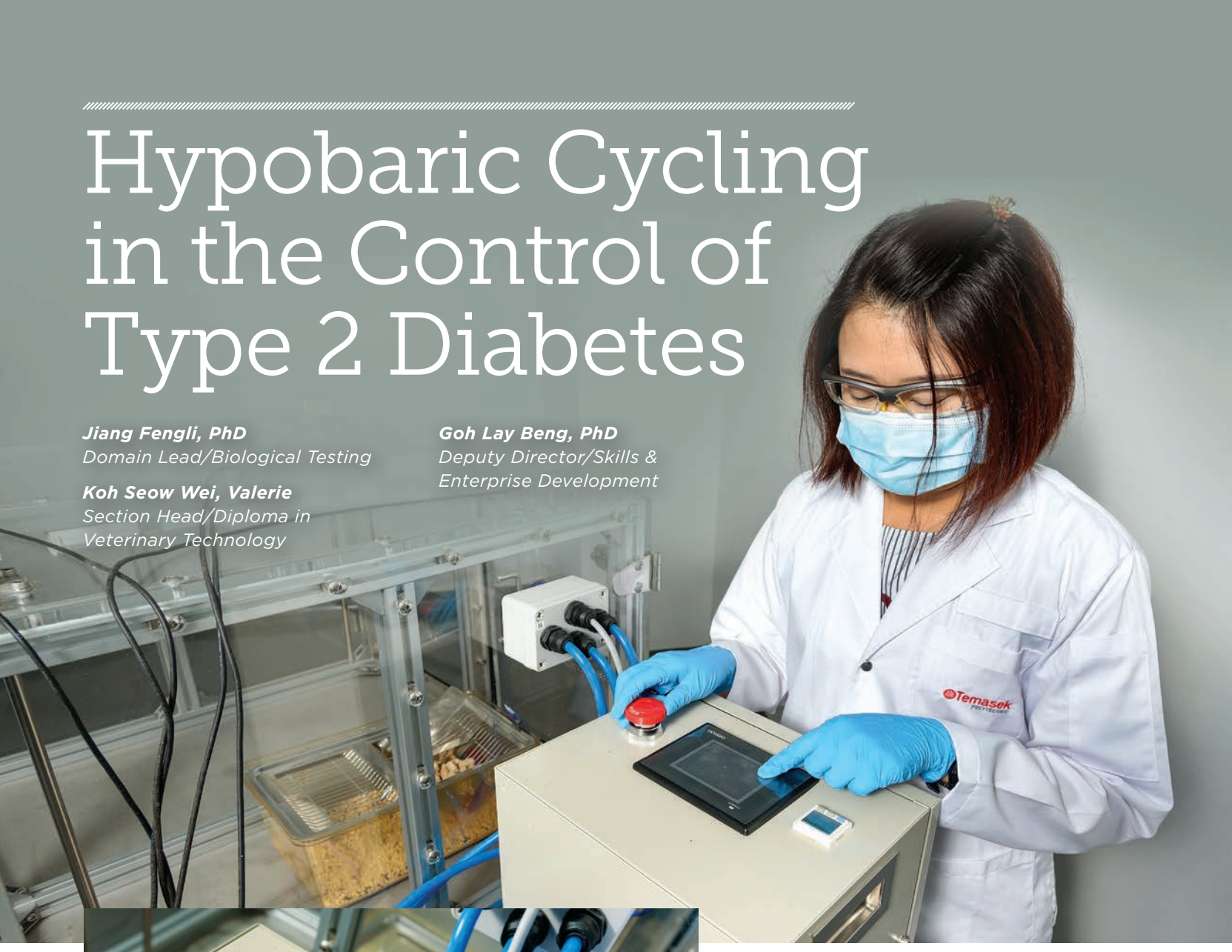
Hypobaric Cycling in the Control of Type 2 Diabetes

Jiang Fengli, PhD
Domain Lead/Biological Testing

Goh Lay Beng, PhD
Deputy Director/Skills & Enterprise Development

Koh Seow Wei, Valerie
Section Head/Diploma in Veterinary Technology

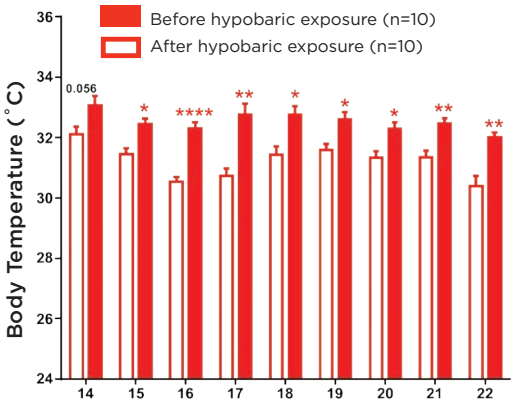


Type 2 Diabetes (T2D) affects nearly 5% of the worldwide population, resulting in huge economic cost. Singapore has the second-highest proportion of diabetics among developed nations. According to a report from the Ministry of Health, diabetes affects 10% of those aged between 18 and 69.

In collaboration with Kei-Y Corporation (Japan), a research team from the School of Applied Science conducted a study to test the effects of cyclical hypobaric chamber exposure in the control of T2D in mice model, a new initiative for the control of T2D. The study was based on preliminary clinical data from Japan, where human subjects, when exposed to similar cyclic hypobaric condition for a few weeks,

demonstrated improved health conditions in T2D. In this study, a high fat diet (HFD)-induced T2D mice model was used. HFD-treated mice presented a clear phenotype of significant high fasting blood glucose (FBG), demonstrating that diabetic murine model was successfully established. These animals were exposed to hypobaric cycling eight weeks after starting on HFD, using the pressure chamber supplied by Kei-Y Corporation. The preliminary results showed that hypobaric exposure, at the parameters as advised by Kei-Y (frequency: 3 min/per cycle; target altitude 1000 meters above sea level; 6 hours/per day), had no significant effects on the mean FBG and glycated haemoglobin (HbA1c) level, up to the period investigated (8-10 weeks). Nevertheless, such intervention was observed to briefly raise the body temperature of the mice by about 10C and slow down the body weight gain induced by HFD (compared to controls fed with HFD). The observed effects on body temperature and body weight gain are consistent with anecdotal human data provided by Kei-Y Corporation. Further investigations are in progress.

This project was presented as a poster and won 2nd Prize at the 7th Asian Federation of Laboratory Animal Science Associations (AFLAS) Congress 2016 held on 7 to 11 November 2016.



*,**,***P<0.05, 0.01, 0.0001, Paired t-test vs. before hypobaric exposure

Figure 1: *Body Temperature*

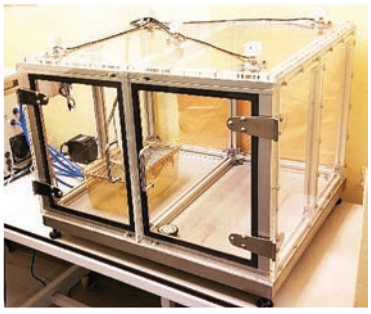
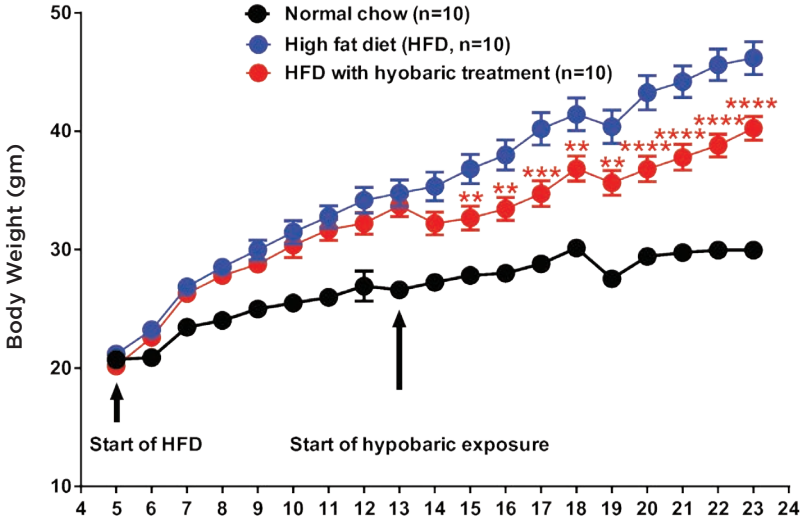


Figure 2: *Hypobaric Chamber*



*,**,***P<0.01, 0.001, 0.0001, 2-way ANOVA vs. High fat diet group

Figure 3: *Body Weight*

Figure 4: *Body Temperature*

"Focus" Mode of Integrated Biomarkers:

A new method to evaluate the dose-effect relationship of TCM formulations

Mohamed Shirhan Bin Mohamed Atan, PhD
Associate Lecturer/Agrotechnology

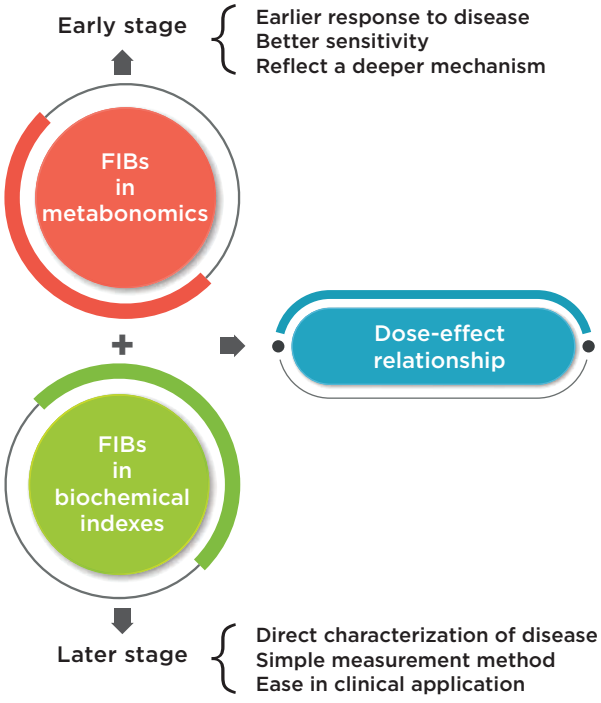
In past decades, substantial efforts have been made to understand the dose-effect relationship of Traditional Chinese Medicine (TCM). The main reason is that TCM has a mixture of characteristics for its multiple components.

It is difficult to accurately evaluate TCM drug efficacy because of the complexity of dose-effect analysis. To date, no specific method for exploring the TCM dose-effect relationship have been developed.

The "Focus" mode, a new tool for research on the dose-effect relationship, is able to identify potential biomarkers applicable for analysing metabolomics parameters and biochemical indexes.

The "Focus" mode accurately focused on a key set of biomarkers - Focus Integrated Biomarkers (FIBs), thus avoiding a great deal of effort associated with the identification of all metabolites.

This provides a new research method for the establishment of dose-effect relationships and for further research on the pharmacodynamics mechanisms of compound medicine.



This highlights the importance of the "Focus" mode and FIBs for accurately representing the effectiveness of TCM in metabolic diseases.

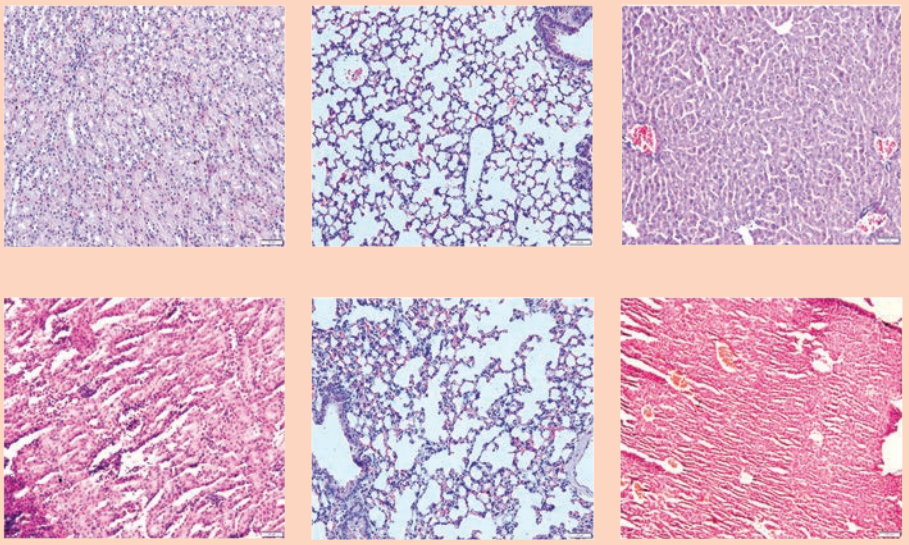
This research not only yields new insights into the evaluation of the dose-effect relationship of TCM in other cardiovascular and neurological diseases, but also provides the possibility of introducing FIBs to the analysis of Western compound medicines.

The main area of research being conducted in Temasek Polytechnic is in metabolic diseases or metabolic-related diseases. This involves testing a drug, fingolimod, FTY720 (FDA approved and prescribed to multiple sclerosis patients), by repositioning it to treat different diseases such as sepsis. We investigated its effect on multiple organ injury in mouse cecal ligation puncture (CLP) polymicrobial sepsis model. The inflammatory markers detected are IL-β, IL-6, MCP-1, MIP-2, TNF-α. Based on the histopathological analysis, we found that the non-selective S1P agonist FTY720, reduces mortality under sepsis in the mice in a dose with no apparent adverse effects to the organs (shown below).

The other metabolic disease that we are working on is Type 2 Diabetes in a rat model using streptozotocin and fed with a high fat diet. We would be using Alpinia Oxyphylla Fructus (AOR), a TCM product used in treating diarrhoea and other related gastrointestinal syndrome in its protective effects on inflammatory status in diabetic rats.

In both models, we utilised known compounds, either western medicine or TCM products, to treat different diseases with the same therapy.

We are trying to narrow down on the known biomarkers that are involved in these metabolic diseases. We would be interested if there were any common biomarkers between these two diseases.



FTY720+CLP *Traces of macrophages. Slight traces of blood in the intracellular spaces.*

Cecal ligation puncture (CLP) *Infiltration of macrophages. Presence of blood in the intracellular spaces in lung and kidney.*

As recent as 2013, the concept of an "integrated biomarker system" (IBS) was proposed by Prof Luo to overcome the issue of lack of focus in systems biology research. Based on the establishment of an IBS, the "Focus" mode of big data analysis was proposed to solve the above mentioned limitations. We would like to apply this methodology in our current metabolic rodent models in sieving out the biomarkers involved based on the treatments given.

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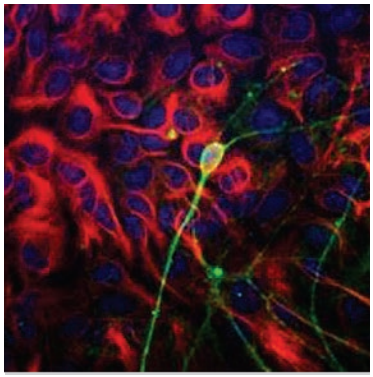
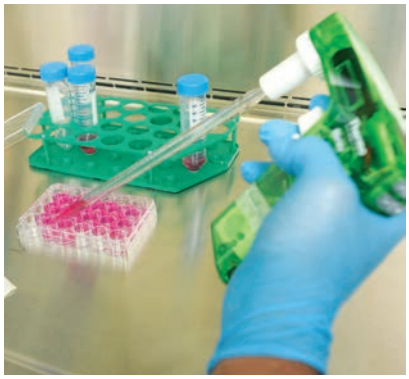


Immunocytochemistry staining of cells



In vitro toxicity testing in primary cells

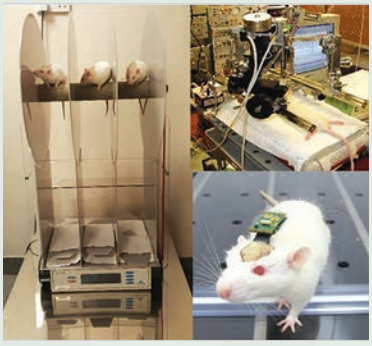
Tissue processing for histology

Real-time PCR analysis of samples

In vitro capabilities:

- Tests for immunomodulatory, anti-inflammatory, anti-microbial, etc.
- Allerginicity/hypersensitivity testing
- Toxicity & mutagenicity response in cell cultures
- Pyrogen testing
- Biomarker analysis for immune responsiveness
- Genetic analysis
- Custom design of testing approaches
- Efficacy testing & validation of products

Lab animals for in vivo testing



Animal model for studying effects on cognition

Hypobaric treatment effect on mice

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  - IBS, sepsis, wound & burn model
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  - Neuroscience - sleep architecture
  - Depression, pain, anxiety & Alzheimer's
  - PK/PD analysis of products

Our facilities

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- Cell culture facility
- Laboratory animal facility
- Biosafety containment facility

For more information, please contact:

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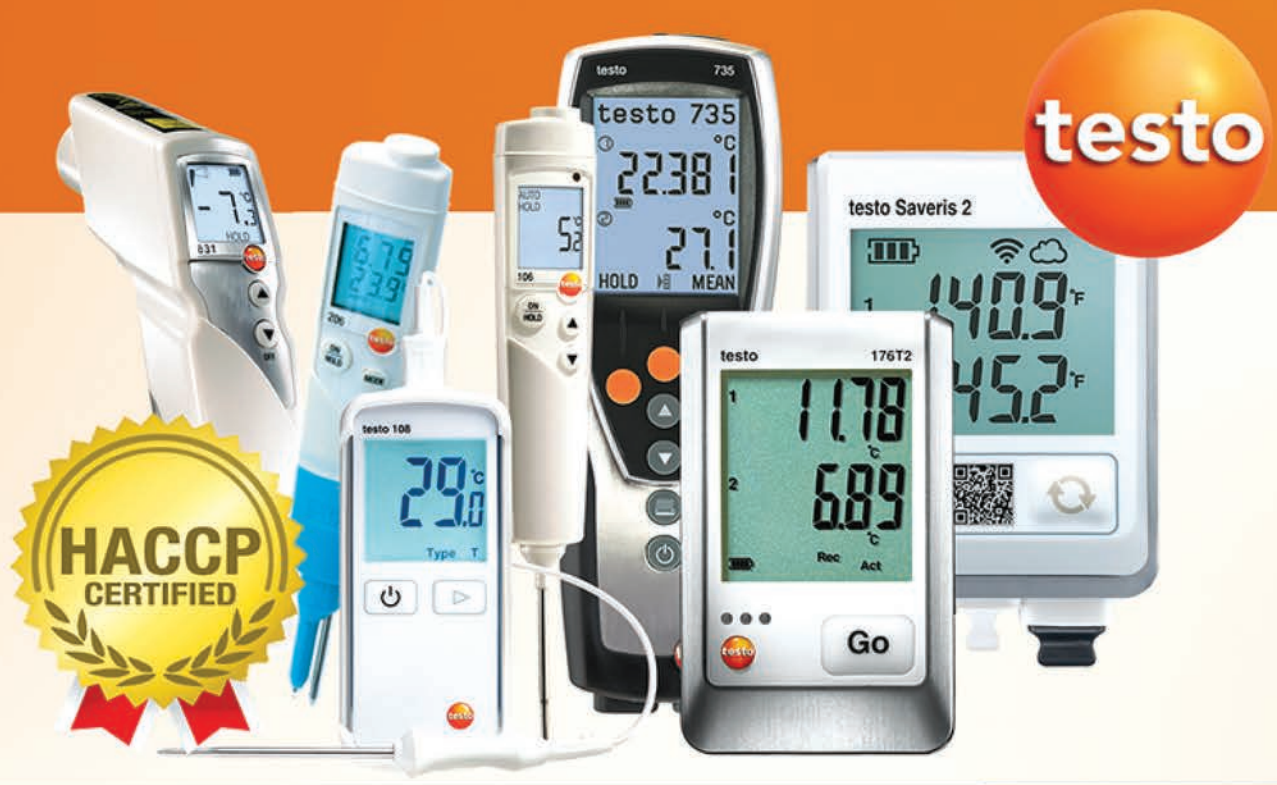

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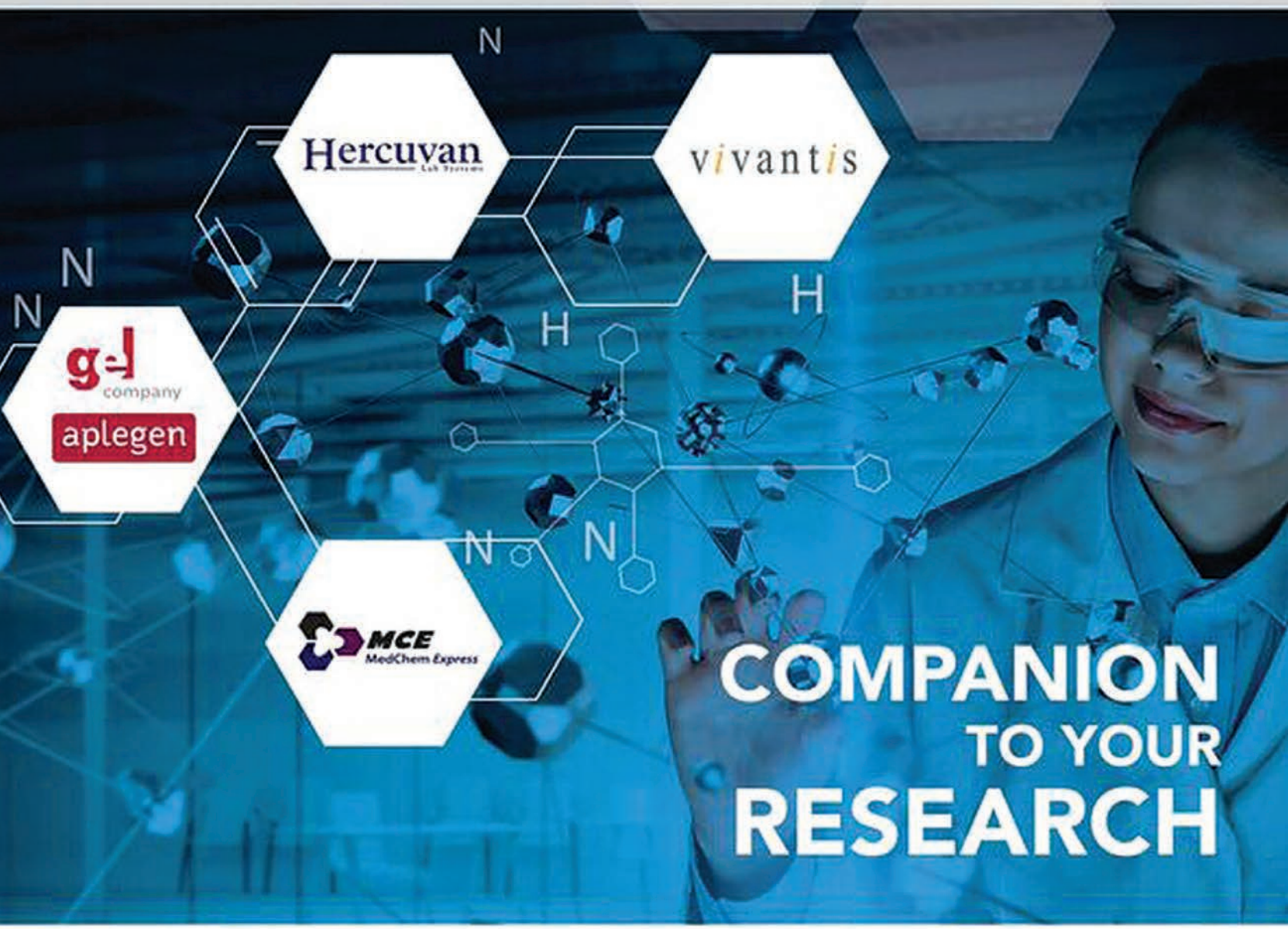

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Yeast Metabolites for Pest Control of Cockroaches

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Section Head/Diploma in Biotechnology

Cockroaches are among the most persistent pest found around the world. They are known to be second only to termites when it comes to controlling their spread in the pest control industry. In Singapore, cockroaches remain one of the most common unhygienic scavengers lurking around garbage chutes of high-rise buildings, old and dirty kitchens as well as dirty sidewalks.

At ASC, an innovative solution known as "TPest" has been formulated to control the spread of cockroaches. A natural cockroach attractant, TPest is made from yeast metabolites, molasses and rice flour. The yeast was initially isolated from fruit peels. During fermentation, the yeast produces volatile metabolites through naturally occurring biochemical reactions. These volatile metabolites are cockroach attractants, useful for luring cockroaches.

For ease of use, the formulation was developed in the form of a "cookie" (Figure 1). The TPest cookie can be placed on a sticky trap as bait to trap unsuspecting cockroaches. As shown in Figure 2, the volatiles released from TPest will attract the pest, rendering them irreversibly immobile on the sticky trap. Since the attractant and trap are biodegradable, this can then be disposed of as domestic waste. Besides cockroaches, TPest can also attract ants, lizards and rats.

In 2016, TPest won the Merit Award at the Tan Kah Kee Young Inventors' Award competition. Moving forward, the team seeks to work with an industrial partner to better develop, as well as to increase the effectiveness and shelf-life of TPest.

Figure 1: *TPest in the form of a "cookie" containing yeast metabolites as attractant*

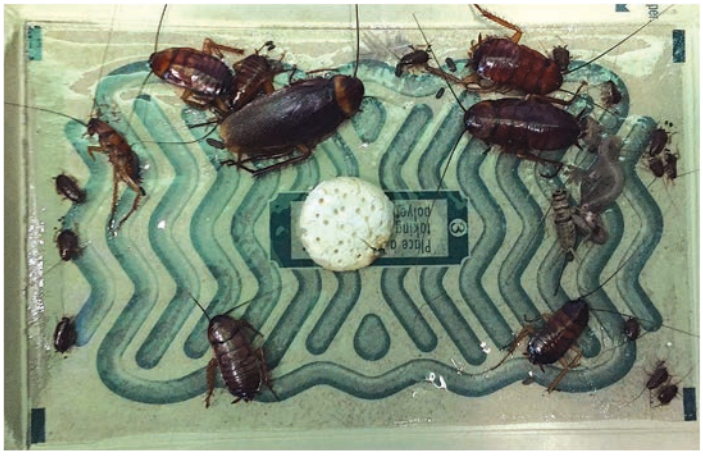

Figure 2: *Attraction of cockroaches to a TPest "cookie"*

Anti-microbial Activity of Organic Liquid Toothpaste

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Lecturer/Biological Testing

Brushing of teeth using toothpaste is the most commonly practised form of oral hygiene. Dental caries, periodontal disease and gingivitis are prevalent worldwide. Most of the oral conditions are caused by bacteria. The success of any toothpaste lies in its ability to eliminate oral pathogens.

The aim of this study is to investigate the antimicrobial efficacy of 100% organic liquid toothpaste in *in vitro* selected test microorganisms. Minimum inhibitory concentration (MIC) of the products were determined by broth microdilution method. The organic liquid toothpaste and two commercial toothpaste products, Sensodyne and Colgate; were tested against the five selected oral pathogens. Standard oral antibiotics (e.g. Chlorhexidine dihydrochloride, CHD) were used as positive controls. The lowest concentration of the toothpaste that prevents visible growth was regarded as the MIC (minimum inhibitory concentration).

The study provided informative data on the organic liquid toothpaste formulation effectiveness in controlling oral pathogens compared to commercial toothpaste containing synthetic antimicrobial agents. Future direction of the project will include investigating the effects of liquid organic toothpaste on normal dental microflora.

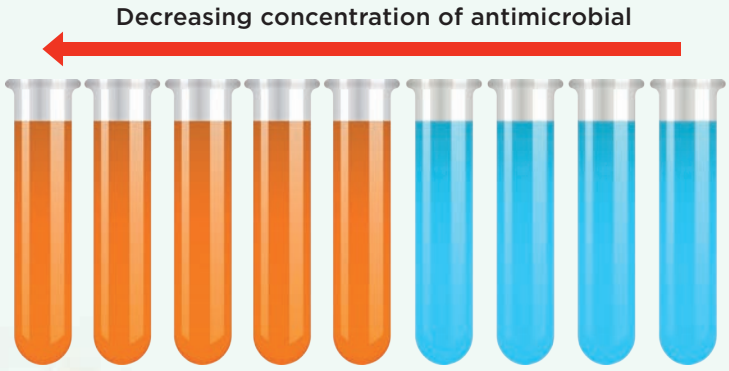
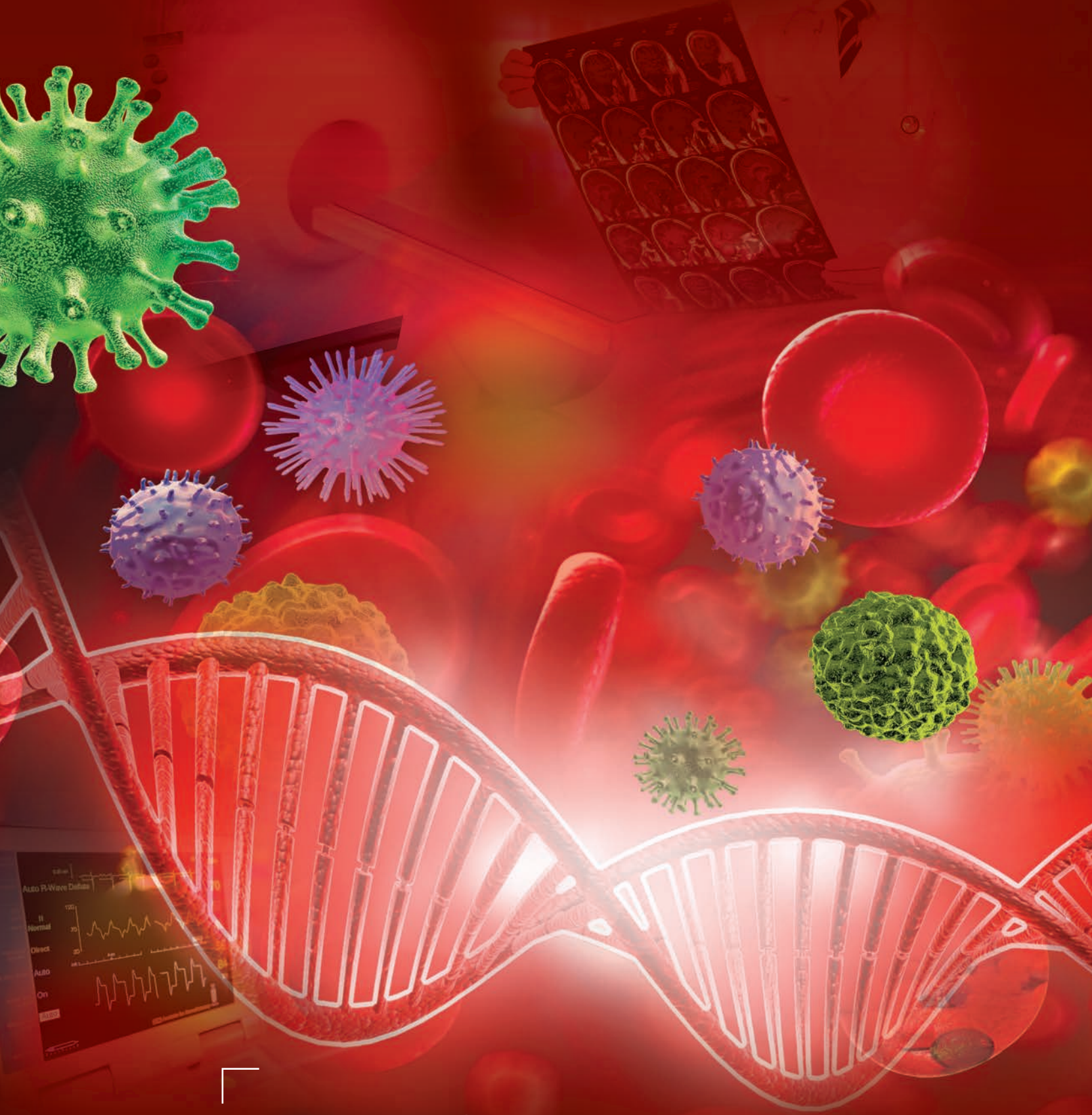


Figure 1: *Broth microdilution assay results used to determine the MIC*



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