Implen Journal Club | November Issue

Welcome to our November issue of the #Implen #JournalClub in 2022.
Awareness Edition

The first issue of the Implen NanoPhotometer Journal Club is giving attention to Diabetes Awareness Month highlighting the exciting work of Alasmar et. al. who recently published in the journal of Molecular Nutrition and Food Research exploring how the composition of the gut microbiota is influenced by sugar. Sugar has been linked with many metabolic health disorders such as heart disease, metabolic syndrome, immune disorders, and diabetes. Evidence suggests that gut microbiota play an essential role in metabolic, nutritional, physiological, and immunological processes with long-term consumption of sugar influencing the landscape of gut

microbiota by altering the gut microbial population called dysbiosis.

The findings of this study show that the high sugar diet influences the composition of the gut microbiome and increases the abundance of microbes associated with the development of obesity. Also, the role of beneficial gut microbes towards a healthy life and the selection of bacteria is driven by the diet and the food available to the gut microbiota. As a result, selected bacteria can control the host feeding behavior to enrich and increase their fitness. Microbes in the gut can induce cravings for specific diets that are optimal for their growth. In addition, it was shown that altered gut microbial flora can be restored with the intervention of healthy food with the rapid reappearance of essential microbes and response of the gut microbiota to dietary impact; with alterations observed within 4 weeks. This important work indicates that the intervention of a healthy and nutritious diet influences gut microbes and this can be beneficial in reducing the implication of early life metabolic disorders and a better understanding of the gut microbiota population.

The quantity and quality of the extracted DNA analyzed using an IMPLEN Nanophotometer®.

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Next issue is continuing with the topic of Diabetes Awareness Month by discussing the recently published paper by Bridge-Comer et. al. which is demonstrating that artificial sweeteners may not represent beneficial substitutes to fructose during pregnancy, with the potential to increase the risk of type 2 diabetes mellitus (T2DM) post-pregnancy. The association of sugar sweetened beverages with increased risk of obesity, insulin resistance, and T2DM is well established and as rates of obesity, diabetes, and related comorbidities have increased, the consumption of artificial sweeteners as sugar substitutes has also risen in popularity as they are perceived as a healthier alternative to sugar sweetened products.

As such, given the propensity of sugars including fructose to induce negative changes in metabolic and reproductive health during pregnancy, the use of artificial sweeteners has been promoted as the ‘healthier’ alternative. This is despite conflicting evidence in non-pregnant

human and animal studies suggesting that artificial sweeteners contribute to metabolic and reproductive dysfunction, and studies during pregnancy implicating artificial sweeteners in the inducement of glucose intolerance and further pregnancy complications. Increased glucose intolerance as well as increased adipocyte size was evident in both artificial sweetener and fructose groups postpartum. As such, the present study adds to the experimental evidence to date suggesting that artificial sweetener consumption over pregnancy and lactation may not be beneficial alternatives to sugar sweetened products and emphasizes the need for more research to be done with regard to this dietary component.

The Implen NanoPhotometer® N60 was utilized in this study to assess RNA prior to being used for cDNA synthesis.

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Next issue is bringing attention to Lung Cancer Awareness Month by highlighting the recent work of Alexandre et. al. in the International Journal of Molecular Science in which they developed a molecular beacon (MB)-based miRNA detection strategy for non-small cell lung cancer (NSCLC). Lung cancer (LC) is the leading cause of cancer-related death worldwide. Although the diagnosis and treatment of non-small cell lung cancer (NSCLC), which accounts for approximately 80% of LC cases, have greatly improved in the past decade, there is still an urgent need to find more sensitive and specific screening methods. Recently, new molecular biomarkers are emerging as potential non-invasive diagnostic agents to screen NSCLC, including multiple microRNAs (miRNAs) that show an unusual expression profile.

Moreover, peripheral blood mononuclear cells' (PBMCs) miRNA profile could be linked with NSCLC and used for diagnosis. In this work, the developed MB approach has shown the potential for miR-21-5p detection in PBMCs from clinical samples towards NSCLC. Following screening PBMCs for the expression profile of a panel of miRNA, a MB targeting of up-regulated miR-21-5p was designed. The results of this study constitute prominent advances towards miR-21-5p in NSCLC with the novel developed MB-based methodology resulting in fast, less

expensive and easy to assess results in NSCLC clinical samples which could constitute a valuable approach for a more specific NSCLC diagnosis.

In this study, the RNA concentration was measured with the Implen NanoPhotometer®. The 260/280 ratio was used to represent protein contamination, wherein values superior to 1.7 are good and related with no protein contamination.

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This issue is highlighting Antibiotic Awareness Week as this topic is becoming a major public-health concern as it is undermining our ability to treat the ever-increasing range of infections. Antibiotic resistance approximately kills 700,000 people each year worldwide, and some experts predict that a continued rise in resistance would lead to 10 million people dying yearly, by 2050. Many studies addressed the role of irrational use of antibiotics as the main determinant of the spread of microbial resistance but fewer focused on the possible effect of the use of non-antibiotic antimicrobial agents. Non-antibiotic antimicrobials, such as chlorhexidine (CHX), are used as antiseptics and disinfectants as well as a component of commercial soaps and detergents. Their use can select resistant mutants with cross-antibiotic resistance, which has led to the FDA suspending the use of certain non-antibiotic antimicrobial agents in medicated soaps, based on their ability to trigger cross-antibiotic resistance. CHX is one of the most widely used biocides among antiseptics and disinfectants which is now used in many healthcare products.

This study demonstrated that CHX exposure, either single or repeated, leads to the evolution of antibiotic-resistant Pseudomonas aeruginosa, a member of the “ESKAPE” group of microbes and is one of the most common microbial pathogens originating in a hospital setting with high mortality rates especially in critically ill and immunocompromised patients. It is an opportunistic human pathogen characterized by an intrinsic resistance to multiple antimicrobial agents, leaving only few treatment options and representing one of the greatest therapeutic challenges. In light of the findings of this study, special protocols should be considered to be implemented during the use of CHX containing preparations and the benefit versus risk for personal-care products

containing CHX needs further assessment.

In this study, the concentration and purity, of the resultant cDNA, were evaluated using Implen NanoPhotometer®.

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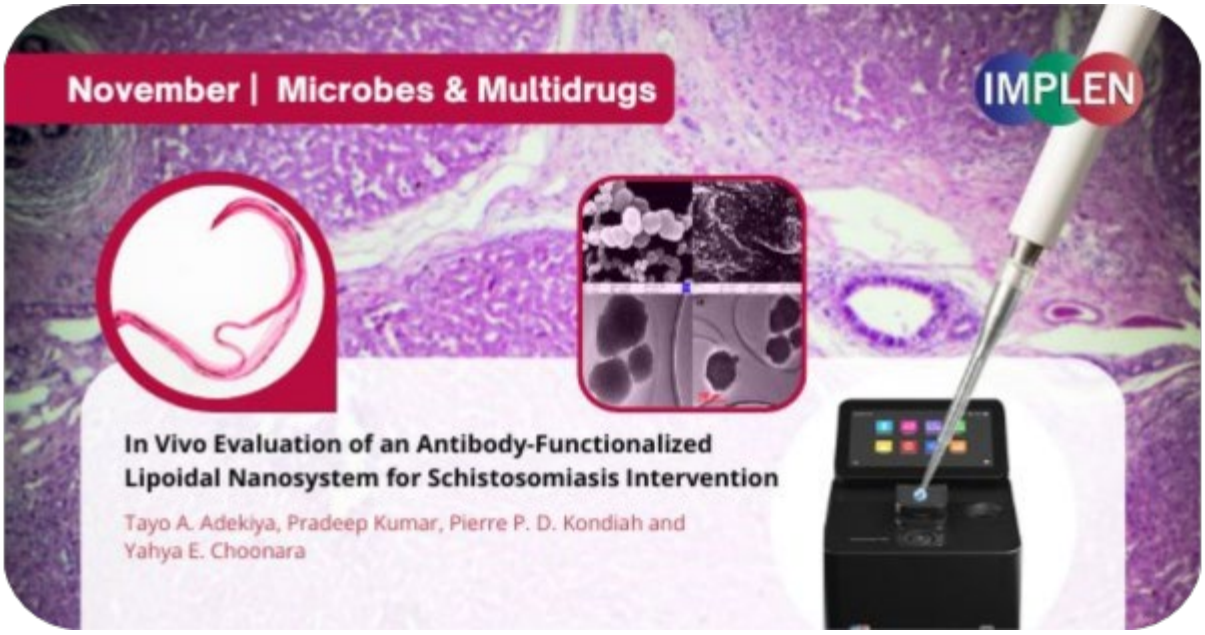

The last issue is continuing on the topic of microbes and multidrug resistance in the context of Neglected tropical diseases (NTDs)- groups of disabling, chronic, and disfiguring diseases that have been abandoned in favor of other well-known diseases because they are most prevalent in poverty extreme areas. Schistosomiasis is one such acute and chronic disease caused by parasitic worms. Adekiya et. al. published a study to evaluate an antibody-functionalized lipoidal nanosystem for Schistosomiasis intervention. Nanotechnological techniques were employed to develop a praziquantel nanoliposomal (NLP) system and surface-functionalized the NLP with anti-calpain antibody (anti-calpain-NLP) for targeted praziquantel (PZQ) delivery. Anti-calpain-NLPs were prepared and validated for their physicochemical parameters, toxicity, drug entrapment efficiency (DEE), drug loading capacity (DLC), drug release, and parasitological cure rate.

The findings of this study revealed that anti-calpain-functionalized nanoliposomes might be used to improve the transport of PZQ into the liver and intestines for targeting both the young and adult schistosomes for schistosomiasis treatment. In addition, the ability of oral anti-calpain-NLP to target young and adult schistosomes in the liver and porto-mesenteric locations, resulting in improved effectiveness of PZQ, hence being a promising therapeutic strategy against schistosomiasis.

The NanoPhotometer® was utilized in this study to measure the antibody coupling efficiency (ACE) on the surface of the nanoliposomes using 260 nm wavelength. The ACE value validated the total quantity of antibodies that was attached to the surface of the nanoliposomes, and it was calculated using the following equation: ACE% = Aq/Tq × 100, where Aq is the actual amount of

antibody attached to the surface of the nanoliposome and Tq is the theoretical amount of antibody employed during the coupling procedure to synthesize the antibody-functionalized nanoliposomes. The NanoPhotometer was also employed in this study to analyze drug (PZQ) release using 263 nm. The amount of PZQ released was calculated from a PZQ standard linear curve (R2 = 0.99).

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