Implen Journal Club | June Issue

Welcome to our June issue of the #Implen #JournalClub in 2021.

Anna-Lena Altenhoff, Sven Thierbach and Alexander Steinbüchel from Westfälische Wilhelms-Universität Münster have recently published their study about enzymatic degradation of rubber with the latex clearing protein Lcp1VH2 as an environmentally friendly and economical solution to treat

the enormous amount of rubber waste. The spectrum of the purified Lcp1VH2 was measured with the NanoPhotometer® to quantify the yield of the protein, as well as to verify the differences in the heme content of the protein. Therefore, the absorbance of the purified protein at 280 nm and the absorbance of heme b at 414 nm was put in ratio to calculate the occupancy of Lcp1VH2 with heme b.

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The global spread of SARS-CoV-2, an infection ranging from asymptomatic to severe with lingering symptomatology in some, calls for a thorough molecular understanding of the pathophysiology of the disease. Symptomatic SARS-CoV-2 patients have increased levels of pro-inflammatory cytokines reported coincident with disease. This prompted the study recently published in Scientific Reports highlighted in this week's hot topics: pathogens issue of the Implen NanoPhotometer® journal club, which was specifically designed by Hye Kyung Lee et al. to fill a gap in the understanding of immunological outcomes in asymptomatic individuals following a community super-spreading event. Residents from the ski resort of Ischgl that experienced a superspreading event of the SARS-CoV-2 infection participated in a study to evaluate whether or not asymptomatic SARS-CoV-2 results in measurable immune activation post-infection, providing critical information on dysregulated immune-response signatures that might foretell disease trajectories. Immune activation following asymptomatic SARS-CoV-2 infection was characterized through a comparative investigation of the immune cell transcriptomes using unbiased RNA-seq analyses on RNA extracted from PBMCs. This study of whole blood transcriptomes identified individual immune profiles within a community population and showed that asymptomatic infection was not associated with enduring immunological activation; thus demonstrating that the use of RNA-seq from mononuclear cells whole blood transcriptome experiments are a valuable tool to monitor immune profiles in community-based cohorts, providing in depth molecular information for significant proportions of the population. The NanoPhotometer® was used to analyze each sample in this study for RNA concentration.

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Pathogens issue of the Implen NanoPhotometer® Journal Club continues with the topic of antimicrobial resistance (AMR), an increasingly global health concern estimated to cause 10 million deaths annually by 2050. In recent years, studies have demonstrated that airborne ARG-carrying human pathogenic bacteria are closely associated with multiple respiratory diseases and infections which can lead to antimicrobial resistance and potential health risk. In modern society, increasing individuals are staying indoors more, which prompted the collaborative investigation published earlier this year in Environmental Pollution led by Yanhui Zhao, QingWang, Zeyou Chen, Daqing Mao, and Yi Luo regarding the exposure to airborne ARGs in indoor environments and the associated risks. In this study, the distribution and composition of aerosol-associated ARGs, bacterial microbiomes, and their daily intake burden in dormitory, office, and outdoor environments was investigated in a university in Tianjin. The results showed that there is a significantly higher abundance of airborne ARGs and bacteria indoors than the outdoors; both of which greatly exceeded the levels reported in other studies, and that the airborne bacterial microbiome could drive site-specific aerosol-associated ARGs. Of particular concern is the wide prevalence and greater diversities of beta-lactam resistance genes in indoor aerosols, which confer resistance to the “last-resort” life-saving antibiotics. The NanoPhotometer® N60 was utilized in this study to check the DNA concentration and quality.

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In this issue of the Implen NanoPhotometer Journal Club Hot Topics: Pathogens, we are continuing on the subject of SARS-CoV-2, a member of the Coronavirus family belonging to the Betacoronavirus genus, the same genus as other notable human pathogens including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus. In an effort to rapidly develop and/or repurpose safe and effective drugs to treat SARS-CoV-2, Alistair S. Brown, David F. Ackerley and Mark J. Calcott at the School of Biological Sciences in New Zealand developed a high-throughput screen using a protein-based FRET-biosensor to identify inhibitors of SARS-CoV-2 that is cost effective and robust with high reproducibility. Their work, which was recently published in Molecules, demonstrated the utility of this biosensor for inhibitor discovery by screening 1280 compounds from the Library of Pharmaceutically Active Compounds collection. The screening identified 65 inhibitors, with the 20 most active exhibiting sub-micromolar inhibition. The top hits included several compounds not previously identified that offer promise as new antiviral drug leads. In this study, the protein concentrations were determined by measuring the absorbance at 280 nm using the NanoPhotometer® NP80 and then calculating the concentration based on an extinction coefficient of 49,530 M−1 cm−1 and 33,640 M−1 cm−1.

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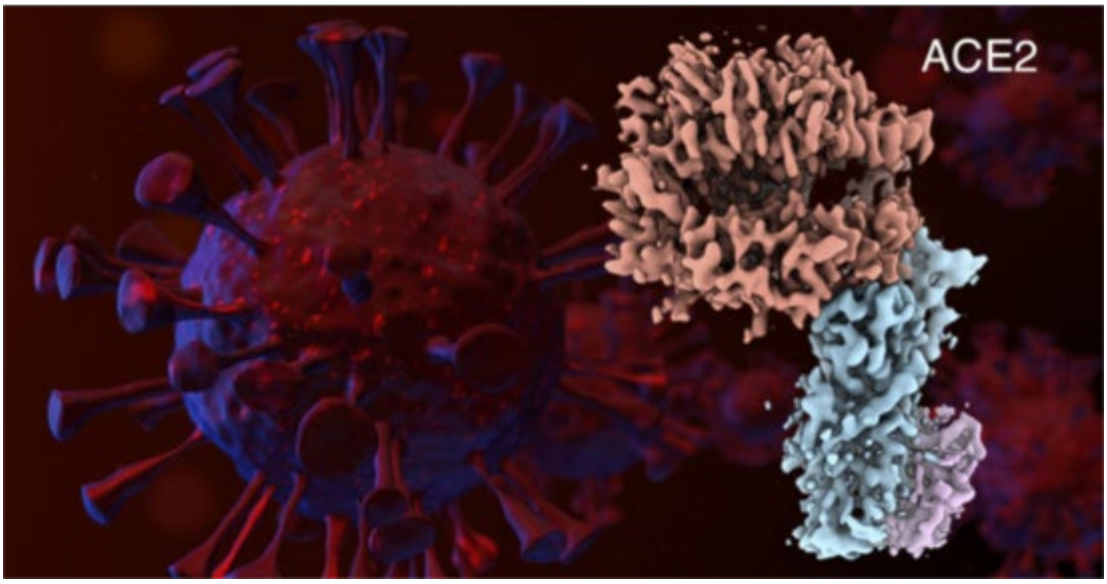

Pathogens issue of the Implen NanoPhotometer® Journal Club continues with the topic of COVID-19. The recently reported new variants of SARS-CoV-2 carrying several mutations in the spike protein were documented in the UK and South Africa. Early epidemiological and clinical findings have indicated that these variants show increased transmissibility in the population and the UK variant of SARS-CoV-2 is thought to be more infectious than previously circulating strains as a result of several mutations. In the April 2021 issue of PLoS Biology, Xing Zhu, Dhiraj Mannar, Shanti S. Srivastava, and Alison Berezuk et al. demonstrated cryo-electron microscopy (cryo-EM) structures of the N501Y UK mutated variant of the SARS-CoV-2 spike protein in complex with ACE2 and 2 potent neutralizing antibodies providing a structural explanation for the increased infectivity. However, this mutation is similar enough in structure for important neutralization epitopes to be retained in the spike receptor binding domain. This was confirmed via biophysical assays and by determining cryo-EM structures of spike protein ectodomains bound to 2 representative potent neutralizing antibody fragments. Protein concentration was measured spectrophotometrically with the NanoPhotometer® N60.

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