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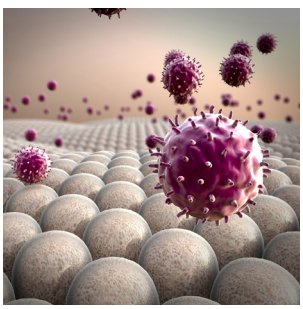
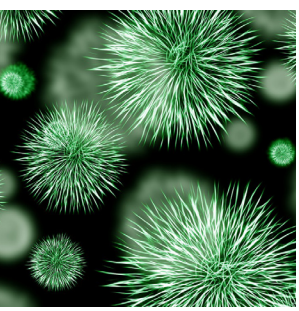
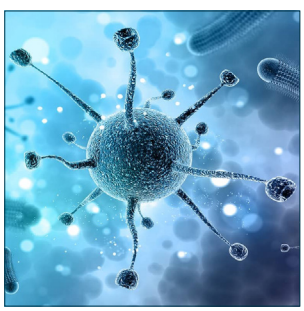
Poster

Clinical Microbiology 2019 & Biotechnology 2019







Joint Event

8th European Clinical Microbiology and Immunology Congress

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3rd World congress on Biotechnology

June 12-13, 2019 | Edinburgh, Scotland

Preparation of natural plants media for the cultivation of lactic acid bacteria and pathogens

Duaa S Al-Dulaimy[1], Basam B Mohammed[2] and **Yusra M B Muhsin[2]**
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Plants play a major role in all the traditional disciplines of medicine because they are considered as a rich source of nutrients. The aim of this study is to prepare an appropriate cultural medium for two groups of bacteria (lactic acid and pathogens). Three types of plants: *Aloe vera*, black and green tea were used. For the preparation of solid media, 100 g/l of each plant were soaked in a warm water, after that 15 g/l of agar-agar were added to each of the aqueous extracts. Furthermore, culture media were prepared by mixing *Aloe vera* with green tea once and with black tea once again. Genera of lactic acid bacteria (LAB) used in this research were isolated from different sources: *Lactobacillus acidophilus* (vagina), *L. casei* (breast milk), *L. paracasei*, *L. plantarum* and *Pediococcus acidilactici* (monkey milk). On the other hands pathogens were *Serratia* and *Pseudomonas* isolated from urinary tract infections (UTI) and *Salmonella* isolated from contaminated food. All test bacteria were cultured on natural medium and incubated at 37°C for 24 h. Study results showed that pathogenic bacteria were not able to grow on black tea medium whereas they could grow on green tea medium and *Aloe vera* medium with few growths on both. LAB showed obvious growth on two types of medium green tea and *Aloe vera*, separately. However, this bacterium could not grow on black tea medium. In terms of the combined plants media, the medium consisted of *Aloe vera* and black tea did not show any growth for LAB but there was a growth of all types of pathogens. Both LAB and pathogens revealed heavy growth on *Aloe vera* plus green tea medium. In conclusion, green tea and *Aloe vera* is a suitable growth medium for LAB while black tea is not appropriate for this bacterium. All types of natural media are suitable for the cultivation of pathogenic bacteria. As a result of the above findings, it is preferred to use natural medium instead of chemical medium that have expensive cost in addition to their negative side effects on human.

Speaker Biography

Duaa S Al-Dulaimy is currently pursuing her PhD in Cardiff University, UK. She has completed her BSc degree from Department of Biology/ School of Biosciences, Mustansiriyah University, Iraq and her MSc degree from the same department. She has published several papers in reputable journals and has been working as a lecturer for more than ten years teaching the undergraduate students of medical microbiology and biotechnology. And, she has supervised several undergraduate students.

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Clinical usefulness of procalcitonin assay to diagnose sepsis: Proposal and evaluation of PCT-qSOFA

Young Ah Kim
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Sepsis is a leading cause of morbidity and mortality, but it is difficult to define sepsis. In sepsis-3, the recent definition, sepsis is defined as an infection-induced long-term failure and the quick sequential organ failure assessment (qSOFA) makes it easy to identify septic patients. However, qSOFA shows a considerable discrepancy, compared with first sepsis definition, which is focusing on abnormal immune responses. The purpose of this study is to improve the low sensitivity of qSOFA. We propose 'PCT-qSOFA' by adding 'procalcitonin (PCT), a useful biological indicator with high sensitivity and specificity to septicemia diagnosis and evaluated the clinical usefulness of 'PCT-qSOFA'.

Total 102 cases with laboratory-confirmed bloodstream infection (BSI) and 102 cases with results of negative blood culture (BC) repeatedly were included for 1 year (2016.5-2017.4). BC, PCT test, qSOFA and systemic inflammatory response syndrome (SIRS) scoring were done in the same day and BSI cases only included definite pathogens *(Staphylococcus aureus, Enterococcus spp. Klebsiella pneumoniae* and *Escherichia coli)*. Total 204 cases were divided to 4 groups such as bacterial sepsis (BSI+ and SIRS+), BC-negative sepsis (BSI-, SIRS+), BC-positive without SIRS and control (BSI- and SIRS-).

In results, PCT alone detects 87.5% of sepsis and qSOFA alone detects 77.2% of sepsis. PCT-qSOFA increased posttest probability (PCT-qSOFA detects 88.4% of sepsis). The area under the receiver operating curve (AUC) was 0.701 for PCT and 0.610 for qSOFA in receiver operating characteristic (ROC) analysis. PCT and qSOFA have prognostic values. qSOFA can be used in ICU patients but the revision of cut-off for qSOFA is needed for best diagnostic performance.

In conclusion, the application of 'PCT-qSOFA' is useful for septicemia diagnosis. We hope that it will help rapid and accurate detection of sepsis.

Speaker Biography

Young Ah Kim is an expert in laboratory medicine. Her main research interests are diagnostic methodology of clinical microbiology and antimicrobial resistance. She is performing studies about an effective diagnostic strategy of infectious disease and transmission model of antimicrobial resistance genes in the community.

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Notes:

Colistin resistant *Acinetobacter baumanii* in a tertiary care hospital of Pakistan: A retrospective single center study

Syed Bilal Tanvir, Ali Shariq and **Hesham S Almoallim**
Dar Al Uloom University Hospital, Saudi Arabia

Statement of the Problem: Multidrug resistant (MDR) *A. baumannii* is a prominent cause of hospital acquired infection. It is a gram-negative Coccobacilli mostly found in soil and is exclusively isolated from hospital environment, but its natural habitat is still unknown. *A. baumannii* is an opportunistic pathogen mostly infecting immunocompromised patients. It can survive on inanimate objects for a long period of time, therefore allowing it to endure in the hospital environment. A surveillance study conducted in the United States revealed that 10% of patients admitted in intensive care unit acquired pneumonia due to *A. baumannii*. The purposes of this study were to determine the resistance pattern of *A. baumannii* to colistin and carbapenem group of antibiotics in a tertiary care hospital located in Karachi, Pakistan and compare it with regional and international resistance pattern.

Methodology & Theoretical Orientation: A total of 705 clinical specimens over the period of July 2016 to June 2017 which included pus, wound swabs, ear swabs, eye swabs, urine, blood, tracheal aspirates and sputum samples were collected. All specimens (wound swabs, ear swabs, eye swabs, sputum, aspirates) were inoculated onto sheep blood agar, cultures were examined macroscopically for growth. Antimicrobial susceptibility was performed by Kirbauer disc diffusion techniques on muller hilton agar plate and standard disc zones were measured according to CLSI guideline. SPSS version 23 was used for statistical analysis. Prevalence and resistance pattern were deduced as well.

Results: A total of 705 clinical specimens were cultured and analyzed for antimicrobial resistance pattern. 61% of the isolates belonged to male patients, while 39% of the isolates belonged to female patients. The isolates were tested against amikacin, aztreonam, sulbactam/cefperazone, ceftazadime, colistin, gentamicin, imipenem, meropenem, tigecycline and piperacillin/tazobactam. 57% and 74% of the isolates were resistant to amikacin and aztreonam respectively. While 60% and 69% of the isolates were resistant to sulbactam/cefperazone and ceftazadime respectively. A total of 14 (2%) isolates out of 705 clinical isolates were also found to be resistant to Colistin.

Conclusion: *Acinetobacter baumanii* isolates show a progressive trend of resistance to carbapenems and colistin. Hence judicious usage of carbapenems and colistin coupled with strict infection control protocols should be implemented. Susceptibility testing for colistin resistance should also be implemented in patients who have gone under treatment with colistin methansulfonate for severe infections.

Speaker Biography

Syed Bilal Tanvir holds an MD degree and a master's in clinical microbiology from Queen Mary, University of London. He completed his postgraduate training in clinical microbiology at Barts and London School of Medicine and Dentistry and the Blizard Institiute. He is currently working in the position of a faculty member at Dar Al Uloom University Hospital, Riyadh, Saudi Arabia. He has previously presented his research in Jeddah, Karachi, Bahrain and London as well. His research interests include surveillance of antimicrobial resistance, systematic review and meta-analysis on newer antimicrobials and surveillance of gram-negative nosocomial infections.

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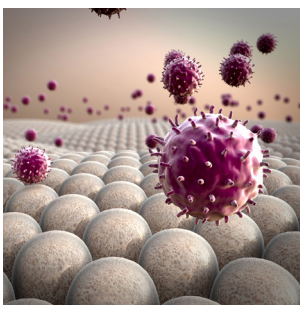

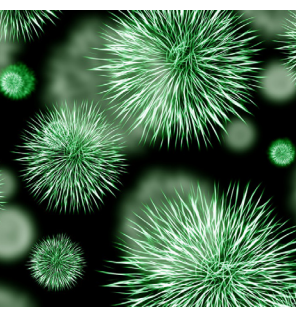

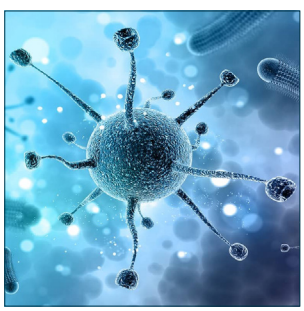

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Efficacy analysis of acid-fast bacillus detection for tuberculosis by smart medical microscope imaging system

Hui-Zin Tu[1,2], Chii-Shiang Chen[1], Huei-Cin Sie[1], Tsi-Shu Huang[1], Susan Shin-Jung Lee[1] and **Herng-Sheng Lee[1]**
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Background: Tuberculosis is an emerging infectious disease worldwide. The most robust and economical method, recommended by WHO, for first line laboratory diagnosis of pulmonary tuberculosis is acid-fast stain method of sputum smears for acid-fast bacilli (AFB) detection. However, it mostly relies on artificial microscopic examination, which may be tedious. The use of such an automated system may significantly increase the sensitivity of bacilli detection. The objective of this study is to adopt an automated system for identification of AFB under microscope using image recognition technology.

Method: The study was carried out in Kaohsiung Veterans General Hospital, Taiwan. An automated microscope system ("system") (TB-Scan 1.0, Wellgen Medical, Kaohsiung) was used in the TB laboratory. The system consists of two components: (1) Microscopic imaging acquiring hardware with auto-focusing and slide-scanning mechanism to cover the specimen based on WHO recommendation (300 fields with 100x oil immersion); (2) Image recognition software for detection and classification of positive AFB in images. The microscopic images were digitally captured and stored. In the detection phase, candidate AFBs were marked and differentiated from other substances in smear based on color and morphological features. In the classification phase, the feature parameters were extracted from AFB candidates as the input parameters to a proprietary classifier. The result was recorded as positive if any AFB was identified in the image of the slide. We used the results with medical technicians as gold standard in evaluating the system performance. Slides with incomplete stain removal and inconsistent viewing fields were excluded from the study (<3%).

Results: When the system was installed in July 2017, the first test results (from July to September of 2017, n=1,050) was not satisfactory. The sensitivity and specificity were only 13.3% (2/15) and 7.9% (73/925), respectively. After a series of customized imaging training and testing, the second test results (from October to December of 2017, n=2,254) were slightly improved: The sensitivity was 28.8% (34/118) and specificity was 53.8% (1,105/2,053), respectively. However, if technicians can be involved in assisting confirming the images to rule out the false-positives along-side with the automated system, the accuracy, sensitivity and specificity can be further improved to 93.0% (2,096/2,254), 67.8% (137/202) and 98.4% (1,959/1,991), respectively. At manufacturer continuous image training by machine learning algorithms, the performance had incremental improvement. For February 2019 only results (n=448), the accuracy, sensitivity and specificity were stability to 91.7% (411/448), 66.2% (43/65) and 96.3% (368/382), respectively.

Discussion: To our knowledge, this is the first of such automated microscope system for TB smear testing in a control trial. Although the performances of the system still have room for improvement, the following issues are worth considering: (1) Medical technicians as gold standard in this study were applied. The system, for example, detected 135 smears positive for AFB but missed by technicians initially but later the final results were reviewed and retrofitted. The result comparisons (technician vs. culture and system vs. culture) may provide more information about the system's performance; (2) A continuous and customized image training for optimizing recognition performance by machine learning is the key to success. TB smear detection is not "one system fits all" and customized training at each laboratory is essential; (3) The inter-laboratory and intra-laboratory variables could compromise the performance of such system. For example, machine staining would be more consistent compared to manual staining; (4) The objective of the automated system is to increase the test performance and not meant to replace laboratory technicians. Experienced technicians are still needed to further improvement of the system.

Conclusion: Microscopic examination by medical technicians is the last mile of the laboratory automation. We believe such automated microscope system could achieve higher laboratory testing accuracy and efficiency worldwide and may have potential to expand to other medical fields such as gram stains, parasite smear and other smears that require labor-intensive works.

Speaker Biography

Hui-Zin Tu is from Kaohsiung, Taiwan. She received her BS degree from graduated from department of medical laboratory science and biotechnology, Kaohsiung Medicine University and MS degree from National Kaohsiung Normal University, Taiwan. She serves in division of microbiology, department of pathology and laboratory medicine, Kaohsiung Veterans General Hospital for more than 20 years with focus on microbiology, especially in tuberculosis. She has published several peer-reviewed articles in identification of *Mycobacterium tuberculosis* using rapid tests and prevention of false-positive results due to NTM in water by a disposable water filters. Her recent research has focused on improving laboratory sensitivity of acid fast stain on mycobacterial bacilla using AI-driven imaging recognition and automated microscopy.

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Accepted Abstracts

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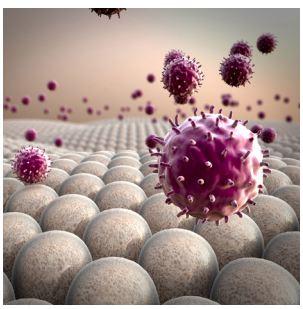

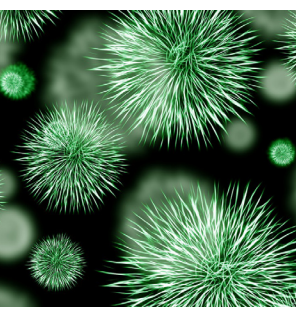

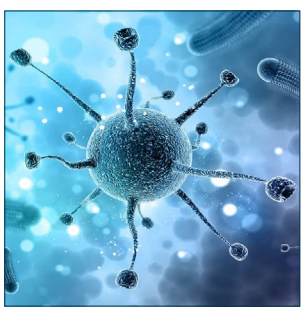

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The Future of medicine: Implantable Sensors

Thomas J Webster
Northeastern University, USA

There is an acute shortage of organs due to disease, trauma, congenital defect, and most importantly, age related maladies. While tissue engineering (and nanotechnology) has made great strides towards improving tissue growth, infection control has been largely forgotten. Critically, as a consequence, the Centers for Disease Control have predicted more deaths from antibiotic-resistant bacteria than all cancers combined by 2050. Moreover, there has been a lack of translation to real commercial products. This talk will summarize how nanotechnology can be used to increase tissue growth and decrease implant infection without using antibiotics but using sensors (while getting regulatory approval). Our group has shown that nanofeatures, nano-modifications, nanoparticles, and most importantly, nanosensors can reduce bacterial growth without using antibiotics. This talk will summarize techniques and efforts to create nanosensors for a wide range of medical and tissue engineering applications, particularly those that have received FDA approval and are currently being implanted in humans.

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Veterinary student opinion on genome-editing in livestock; progression towards a regulatory framework

Oskar Ulvestad
The University of Edinburgh, UK

There is a lack of explicit regulation in the UK (EU) in relation to genome-editing of livestock. Uncertainty of regulatory acceptances makes it hard for research relying on commercial support to get off the ground, hindering development of potentially beneficial applications such as cure or prevention of congenial conditions and disease. The views of various potential stakeholders have been seen to influence policy and regulatory frameworks, for example when GMO crops failed to gain acceptance in Europe. Some (limited studies) have been carried out relating to the attitudes of the public to genome-editing, but not of veterinary students who belong to an important stakeholder group. 27 veterinary students at Edinburgh University answered a questionnaire on issues related to genome-editing in livestock. The analysis of the data collected indicates the respondents are positively disposed to genome-editing in general. The support is strongest when it comes to disease control, and more ambiguous when the aim is to increase productivity. Combined with high levels of public trust in veterinaries, the implications are that they can be useful in progressing the regulatory process by engaging with the public and that the focus of the process should be on welfare rather than solely productivity gains.

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Synthetic life: How to create new organisms from the computer to its industrial scaling

Francisco Cruz Rodríguez
Synbiomics Group, Mexico

Escherichia coli are a very organism important at the biotechnological level due to its many relevant physiological characteristics In addition to its fermentative metabolism gives it a unique potential for industrial biocatalysis. The application of recombinant DNA methods for the expression of heterologous genes in *E. coli* can improve the production of Metabolites and proteins of industrial interest, allowing the introduction of native or non-native metabolic pathways, for the production of a wide range of chemical products. Metabolic engineering has been in recent years responsible for providing strains producers, however, in some cases; metabolic engineering fails to supply good production strains. In recent decades, systems biology and synthetic biology, have allowed us to model and design organisms (synthetic organism) whose phenotypes are evaluated from the computer and not through trial and error (experimentally speaking, as it comes doing with traditional techniques and methods) due to the conception of what is known as Genomic Scale Metabolic Models (Genome-scale metabolic models, GEMs), which is the in silico representation, of all the biochemical reactions that occur in an organism, coding detailed and global information (genomic) on an organism in a computational framework, with the objective of predicting the cellular behavior of a given genotype, under certain restrictions (rate of growth, production yield of the product, carbon source, etc.). Offering a more rational, systematic design. Modeling gen-protein in the computer and genotype-phenotype, you can design production strains in a more smart and efficient accelerating both the yield and the industrial scaling of the production of the metabolites synthesized by these synthetic organisms.

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Design and construction of a genetic vector expressing a poly-miR-122 for gene therapy of hepatocarcinoma

Mariela Montaño-Samaniego
Escuela Nacional de Ciencias Biológicas, México

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide and there is still no effective treatment for this disease, so gene therapy is a promising therapeutic alternative for the treatment of HCC. The use of microRNA (miRNA) in gene therapy has become a powerful tool for the regulation of genes involved in acquired genetic diseases such as cancer. The miRNA-122 (miR-122) is specific and the most abundant in the liver, it has been shown to function as a tumor suppressor. The levels of miR-122 decrease significantly and specifically in HCC. The objective of this work was to construct a genetic vector that contains a poly-miR-122 governed by the α-fetoprotein (AFP) promoter, specific to HCC. A poly-miR-122 sequence containing three miR-122 precursors (pre-miR-122) was designed, this was analyzed for secondary structure prediction and thermodynamic stability. The result of the prediction analysis of the poly-miR-122 sequence showed that the primary transcript will have thermodynamic stability, indicating that it can function in the treatment against HCC cells. Subsequently, the recombinant plasmid pIRES2-AFP-poly-miR-122-EGFP was constructed. The identity of this recombinant plasmid was confirmed by enzymatic restriction. The presence of the AFP promoter in the recombinant plasmid was confirmed by PCR. With automated sequencing, an identity of 99.6% was found with the AFP promoter (NCBI: L34019.1). This genetic construction can express the active and stable miR-122, so it could be used for the specific treatment of HCC by gene therapy.

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Characterization of recombinant plasmid PEPCK-PIRES2-EGFP and evaluation of PEPCK promoter specificity in hepatocytes cells culture transfected with liposomes

Lucas-González Amellalli
Instituto Politécnico Nacional (IPN), México

Gene therapy consists of introducing foreign genetic material, DNA or RNA, into cells with genetic disorder, in order to create beneficial biological effects. In order to achieve this effect, it is necessary to introduce the therapeutic genes through a genetic vehicle. Liposomes are gene delivery vectors with very low immunogenicity and toxicity, ease of manufacture, furthermore, they lack DNA insert size limitation. Cationic liposomes seem to be the most hopeful, due to his ability to interact with the DNA and the cell surface. Once material genetic gets inside cell, it can carry therapeutic genes whose expression is regulated by tissue and organ-specific promoters. PEPCK promoter is stably active and specific in hepatocytes, so it could be adapted to therapeutic interest genes and get into the body for selective expression in liver cells. In this work, the genetic construction pPEPCK-IRES2-EGFP was characterized by enzymatic restriction, PCR and nucleotide sequencing, confirming the presence of the organ specific promoter PEPCK. Once the identity of the genetic construct was confirmed, it was used to transfect the hepatocarcinoma cell line HepG2, the embryonic kidney cell line Hek293FT as a control of specificity and a primary culture of hepatocytes, using cationic liposomes from the DLO and DLE lipids as a delivery vector. The specificity of expression in liver cells was evidenced by the green fluorescent protein gene contained in the recombinant plasmid used. This is intended to create a powerful tool to address the expression of therapeutic genes in the treatment of liver diseases, without affecting other cell types.

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Marine biotechnology as a support for the development of the blue bioeconomy in the EU H2020 framework

Donatella de Pascale
Institute of Protein Biochemistry, Italy

The marine environment includes incredible biological and chemical diversity, which are still largely unexploited. Marine microorganisms possess secondary metabolites that assist in survival and defence in these harsh habitats. Particularly, marine resources are involved in added-value products and processes in the food, cosmetic, pharmaceutical and bioprocess industries. Bioprospecting for these natural products are important to the EU Blue bioeconomy, which is focused on creating employment, boosting economic growth, and contributing to a healthier and sustainable society. Since, the seas and oceans play a pivotal role in driving the EU economy, its contribution to achieving the goals of the EU H2020 strategy for smart, sustainable and inclusive growth cannot be overlooked. However, additional growth can be acquired by developing sectors that have a high potential for sustainable jobs and growth. To fully exploit these promising biological resources, new strategies in the pipeline as well as a new cohort of cross-disciplinary trained scientists are needed to overcome existing bottlenecks for the production of high value biomolecules.

Donatella de Pascale is the coordinator of the following H2020-MSCA projects:

The H2020-MSCA-ITN-ETN: **MarPipe** is a Research and Training Network program of 11 academic and industrial partners based in 8 European countries working in collaboration to train 11 Early stage researchers in the field of marine drug-discovery focused on antimicrobials and anticancer compounds. **The H2020-MSCA-RISE**: Ocean Medicines is a network of academic and SMEs across Europe and Africa, with proven experience in training and endowed with state-of-the art scientific and technical expertise and infrastructures, aimed at fostering young marine biodiscovery scientists from the development of a new drug to its commercialization, innovation and possibilities of entrepreneurship. A general overview of the major achievements obtained from these two project will be given in order to highlight the recent progress in this field.

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The cooperative approach to biotechnology for the promotion of education inclusion, improved agriculture, and science-based industries: An ongoing experiment from a rural area in Argentina

Lentz EM
IdESA-UGACOOP, Argentina

Cities around the world with a rich history of renowned universities have seen the rise of biotech-based companies, which further stimulates the concentration of creative and opportunity-discovering minds in these interdisciplinary centers. In this long-term project, we aim to promote such process in the 50,000-people city of General Alvear in Mendoza, which neither counts with a university, nor a research center, and its main economic activities are based on agriculture. A biotechnology lab has been constructed with funds from the local government, maintained with the support from cooperative energy and wine producing companies in the area, and managed by an interdisciplinary group of professionals. We have started teaching the first year of a biotechnology technical degree making use of both DIY-Bio and low-cost approaches, and we are observing growing interest among students in town and surrounding cities, who are looking for non-traditional career options. A collaboration with the government organization ISCAMEN has been started to produce and commercialize biocontrol agents and insect-derived products to supply the growing demand from organic producers that can access to new markets, created by consumers around the world interested in good agriculture practices and decrease use of phytochemicals. Another key collaboration addresses the need of wine producers of "Algarrobo Bonito" for virus-free plant material. The generation and certification of healthy plants stocks of *Vitis vinifera* by virus specialists in INTA Lujan de Cuyo, led by Dr. Gomez-Talquenca, and the micropropagation of these plants in our cooperative laboratory, will make this high-quality material available for local producers, leading to an upgrade of their wineyards with an associated yield increase in the fields. These "biotechnological seeds" could generate a synergistic critical mass of science-based individual entities, collaborating in a highly mutualistic community of entrepreneurs and academic environment, contributing to the evolution of city development and progress.

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Mixed consent as the prerequisite for the freedom of biomedical research

Edvinas Meskys
Vilnius University, Lithuania

I have defended the PhD thesis in law on the topic *"Mixed consent as the prerequisite for the freedom of biomedical research"* and would like to share my insights in the congress. Research biobanks collect human biosamples and the related information, which in the future can be used for comprehensive scientific research. However, in spite of the undeniably positive effect, one should not ignore the theoretical, practical or even ethical problems arising from the operations of biobanks. Research aim: To find a proper legal regime, which would maximally protect the human rights, but also would not deny the very essence of the research to have an opportunity for a free choice of the scope of the research, the opportunity to experiment, and in this way to make discoveries in the field of health that are important for all the society.

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