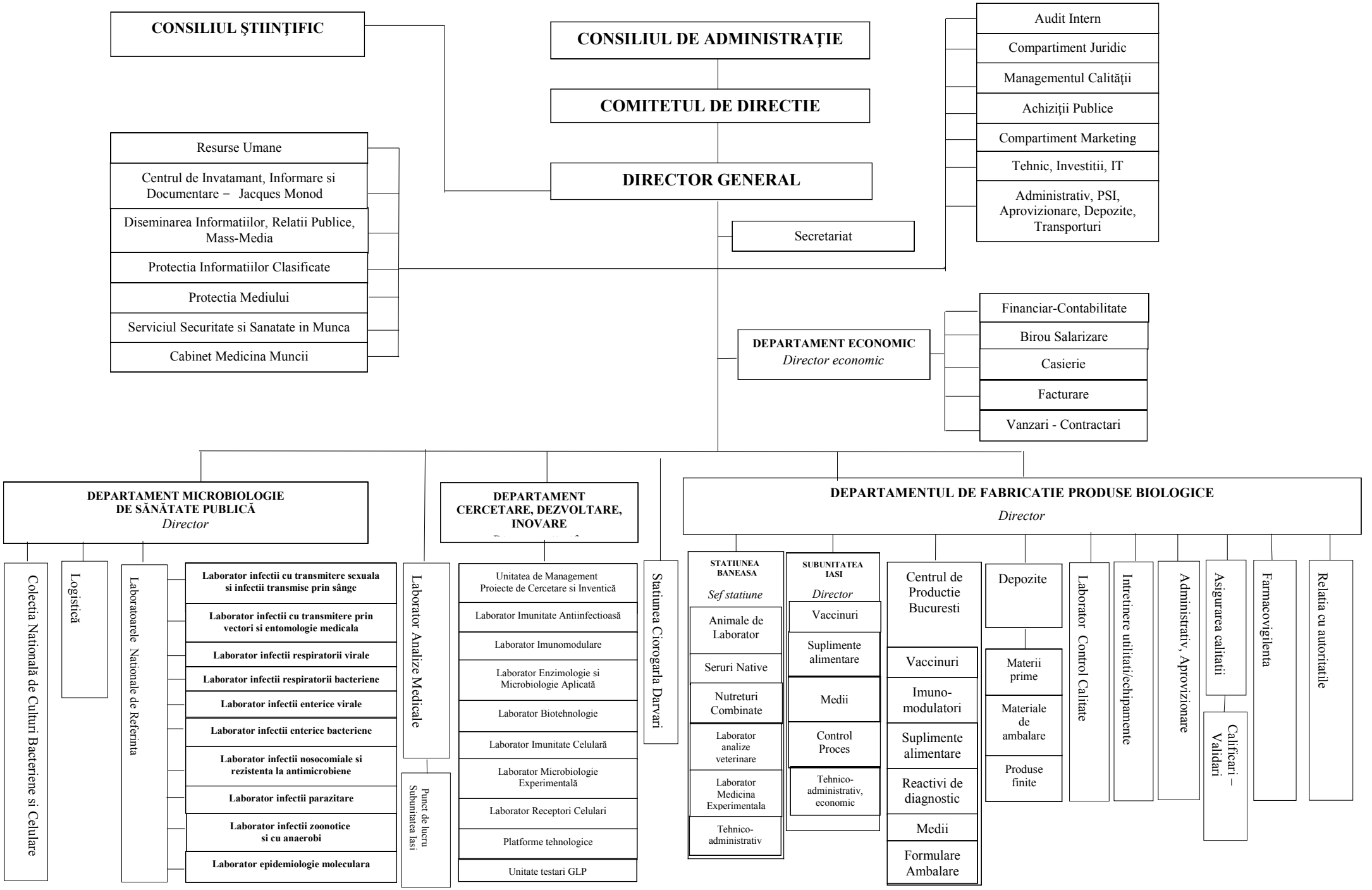
ANEXA

INSTITUTUL NATIONAL DE CERCETARE – DEZVOLTARE PENTRU MICROBIOLOGIE SI IMUNOLOGIE „CANTACUZINO”
STRUCTURA ORGANIZATORICĂ



GENERAL ACTIVITY REPORT OF THE INSTITUTION

The National Institute of Research-Development for Microbiology and Immunology "Cantacuzino" (NIRDMIC) is a national institute of research and development in coordination of Ministry of Public Health. Historically, it was created as a strategic institution to perform research in all domains of microbiology and related sciences in order to develop the production of therapeutic sera and vaccines. Currently, our main fields of activity consist of research in immunology and immune mediated diseases, microbiology, virology, parasitology and other infectious diseases (LS6). Other connected fields such as genomics, molecular genetics, transcriptomics, proteomics, metabolomics, bioinformatics (LS2) and aetiology, diagnosis and molecular epidemiology of infectious diseases, public health (LS7) are also approached in order to accomplish our mission.

The Cantacuzino Institutes mission, as stated by its founder, Prof. Ioan Cantacuzino is to promote public health by high quality and competitive interdisciplinary research, by monitoring, prevention and control of communicable diseases. Accordingly, the following research directions are focusing on:

- Studies regarding the pathogen circulation and their virulence characteristics;
- Emergence of antimicrobial resistance and research for new compounds with antimicrobial activity;
- New methods for diagnosis, immunology and epidemiology of infectious diseases;
- Biotechnology and vaccine development;
- Mechanisms of infections and immunological response;
- Environment changes and vectors disseminated diseases;
- Genomics and proteomics;
- Immune mediated disease mechanisms and therapies;
- Capacity building for technology transfer in vaccine production.

Strengthening the professional and institutional capacity for an effective participation to the national and international programmes for research, control and surveillance of infectious diseases and promotion of national and international co-operation were the main objectives of our activity.

Since 1991, National Institute of Research-Development for Microbiology and Immunology "Cantacuzino" is a member of Institut Pasteur International Network, a partnership of 32 research and public health institutes on five continents.

National research projects

Within the national research programs, PNI and PNII, our institute developed projects of included in calls from IDEAS and Partenerships in Priority Domains programs, mainly within the 4 (Health) and 6 (Biotechnologies) domains. A number of 10 CEEX projects (2005-2008), 16 CEEX projects (2006-2009), 32 projects PNII (call 2007 and 2008), four IDEAS projects (call 2007 and 2008) and one Capacities project (2008) were undertaken by researchers from institute. The research subjects approached in these projects could be summarized as follows:

Communicable diseases, diagnosis and surveillance

- Improve infectious disease diagnostic in order to reduce un-known aetiology infection reports. Design and proposal of diagnostics algorithms based on classical microbiological and molecular methods;
- Studies on virulence molecular markers used for detection and characterization of highly pathogenic as well as commensal/opportunistic bacteria involved in human infectious diseases;
- Optimization of molecular methods used for tracking infectious diseases (microbial genotyping) and approaches based on the innovative concept of monitoring multiple interfaces: human health / animal health / wildlife / environmental;
- Harmonization of laboratory methods with those used by Public Health laboratories for an effective participation in the ECDC programs for surveillance and control of communicable diseases;
- Molecular characterization of emergent, fastidious, un-typable, non-cultivable microorganisms (viruses and bacteria) and evaluation of the impact their variability has on diagnosis and pathogenesis of infections;
- Improve detection of HCV and/or HBV from human patient serum versus hepatic tissue after surgical intervention in order to define the viruses role in carcinogenesis;
- Studies on mechanisms and genetics of antimicrobial resistance (bacterial and viral) and development of new compounds with antimicrobial activity;
- Studies on biofilm and Quorum Sensing mechanisms and their role in infections development/treatment.

Immunology, Biotechnology and vaccine development

- Studies on innate immunity, inflammation and immune regulation;
- Autoimmunity and therapeutic aproaches for autoimmune diseases and allergies;
- Immune mediators profile in human pathology;
- Molecular mechanisms involved in cellular dysfunctions and identification of new therapy for human disorders;
- Diagnosis and identification of new therapeutic targets in communicable and inflammatory diseases;
- Development and implementation of new technologies for cloning and over expression of mammalian cytokines;
- Studies on immunogenicity of avian influenza virus in mice and humans in correlation with administration route;
- Development of cell-based vaccines for influenza viruses using modern technology as a replacement for eggs and chicken embryos;
- Bacterial nucleoside monophosphate kinase family as new therapeutic targets for antibacterial agents;
- Development of monoclonal antibodies (mAbs) and immunoassays for detection of highly pathogenic bacteria and viruses;

Laboratory animals

- Elaboration of standard operating procedures and preclinical study models in accordance to national (ANM) and international (EMEA) rules.
- Establishment of regulations for using animals in laboratory experiments

Collaborations with SME

- Synthesis of therapeutically active biopolymers, using glycerine (by-product of biodiesel manufacture) as a substrate;
- Study of plant extracts with immunomodulatory properties (anti-tumoral and/or anti-inflammatory)
- Development and implementation of original, innovative, competitive products for food packaging (bio polymeric antimicrobial foils), based on biologically active molecules from natural regenerable resources;
- Studies regarding the antimicrobial efficacy of different Copper and Silver metallic nanofilm covered materials and demonstration of their benefit in reducing the microbial flora associated with the hospital environment;

These projects resulted in the creation of national and international multidisciplinary research consortia within our field of activity further improving our competitiveness and visibility and opening new opportunities for research in the following years. As a result, in the 2011 national call for research, NIRDMIC had 11 project proposals as coordinator, six of them being PCCA type 2 projects for collaboration between public sector and industry.

International research collaboration

Collaboration within the Institut Pasteur International Network

As a member of the Institut Pasteur International Network (RIIP), the National Institute of Research-Development for Microbiology and Immunology “Cantacuzino” participates to collaborative research projects within the network (5 projects in the last five years). Current collaborative research projects aim at improving the capacity to detect and characterize aetiological agents involved in different infectious diseases. Research subjects focused on the interrelationship between hepatitis virus genotype and host in the development of primary liver cancer (ACIP A24), role of enteroviruses and poliovirus co-circulation in the generation of recombinants (PTR 276), incidence of antibiotic resistance among pathogens isolated from digestive and urinary tract infections (projects FSP), and use of molecular methods for detection and characterization of pathogens (ACIP 01 2009). These projects created a large opportunity for collaboration between researchers from different continents were Pasteur Institutes are located and created the opportunity for training courses and stages organized in Pasteur Institute Paris or other institutes within the network. During 2007-2011 young researchers from our institute participated in training courses and stages on diphtheria diagnosis and molecular characterization of strains, real-time PCR detection of whooping cough, detection methods for anaerobic bacteria and clostridial neurotoxins and diagnostic and typing of *Salmonella* and *Campylobacter* isolated from food borne infections. Also, as a result of RIIP membership, NIRDMIC

benefits from scientific advisory, two researchers from the Pasteur Institute Paris being permanent members of our Scientific Board.

Collaboration within EU projects

As the sole national vaccine manufacturer, in an era dominated by the threat of new pandemic influenza emergence, the institute was involved in projects regarding the advancement of capacity for influenza vaccine production (WHO project **Pandemic Preparedness Project**) according to EMEA conditions, in order not only to cover the national vaccine needs, but also be eligible for distribution to neighbouring countries. The DG-SANCO project, **Fast VAC** has as main objective to put into effect a comprehensive set of predictive rules enabling accelerated development, evaluation, production and release of emergency vaccines. “Combating flu in a combined action between the industry and the public sector in order to secure adequate and fast intervention in Europe” was the subject of **Flu Secure**, a DG-SANCO project developed during 2006-2010, a collaborative action between NIRDMIC and NIBSC aimed at technological transfer, immunogenicity evaluation of H5N1 influenza vaccines in animal models and building a reagent bank for the multiplication of seed strains in cell culture. “Resistance of Influenza Viruses in Environmental Reservoirs and Systems” (**RIVERS**), a FP7 project developed during 2007-2009, studied the impact of water treatment (addition of chemical compounds, different pH values, salt concentration, and temperature) on H5N1 virus survival. Participation in **ConFluTech project** (Capacity building for the control of Avian influenza through technology transfer and training), a FP6 project, gave the opportunity to participate in training courses and workshops on technological transfer, molecular tools for pathogen detection and discrimination, epidemiological analysis for monitoring and modelling of avian influenza and to outbreak response. In September, a two day workshop (26-27) was organized in our institute, the participants presenting recent findings on the epidemiology of avian influenza in Romania, diagnostic tools and laboratory biosecurity. A collaborative project in the frame of EpiConcept, (**contract OJ/2007/015**) has as main objective monitoring seasonal and pandemic influenza vaccine effectiveness. Recognizing our proficiency into the field, the WHO selected the institute as a WHO Collaborative European Centre, training courses and workshops being performed here for participants from all European countries.

A main direction for research is the eco-epidemiology of vector-borne diseases in the changing European environment which has been developed in the framework of FP6 and FP7 projects: **EDEN** (2005-2009) and **EDENext** (2011-2014).

EDEN project (**FP6, GOCE-CT-2003-010284; Emerging Diseases in a changing European eNvironment**) which included partners from a number of 49 institutions in 24 countries aimed to identify, evaluate an catalogue European ecosystems and enviroenmantal conditions linked to global change which are influencing the spatial and temporal distribution of human pathogenic agents. The project developed and coordinated at the European level a set of generic methods, tools and skills such as predictive emergence and spread models, early warning, surveillance and monitoring tools and scenarios, decision support for intervention and public health policies at both EU and national or regional level. Our research teams participated in three subprojects: tick-borne diseases, West Nile virus, and malaria. The project resulted in the gathering of new to science knowledge on the circulation of West Nile virus, ticks as vectors of Lyme disease and Anopheles mosquitoes as potential vectors of malaria. Three doctoral theses were ellaborated in the framework of this project. The same team of scientists participated in the FP7 call on vectors biology and vector-borne diseases (2010), as

partners, in four project proposals, of which one has been selected for funding: **„Biology and Control of Vector Borne Infections in Europe", acronyme EDENext, GA 261504"** which is be developed in the following years (2011-2014). Cofinancing for this project is supported by 147-1EU /2011 of National Authority for Research. The **EDENext** project addresses biological, ecological and epidemiological components of vector-borne diseases introduction, emergence and spread, and proposes advanced tools for controlling them. The INCDMIC team is involved in the studies of West Nile virus mechanism of pesistence in the European environment via vertical transmission and overwintering of mosquito vectors. Two doctoral fellows are preparing theses in the framework of this project.

Researchers also participated in **ArboZooNet** project, an International Network for Capacity Building for the Control of Emerging Viral Vector Borne Zoonotic Diseases, a Coordination Action funded for 3 years by the European Union (EU) under FP7 that was launched in May 2008. International collaboration enabled study of circulation of high bio-risk pathogens borne by ticks.

ECDC representatives for laboratory surveillance of communicable diseases.

Appreciating the professional expertise of scientific personnel the institute was nominated as competent body to the ECDC for the laboratory surveillance of the infectious diseases. In this position, experts from our institute are national microbiological contact points for communicable diseases included in the ECDC surveillance programmes. They are participating in training courses, workshops, and annual meetings organised by ECDC. Some projects were implemented in order to enhance the capacity of Public health laboratories to detect and characterize aetiologic agents of communicable diseases and to harmonize the microbiological methodologies for a competent participation in the international projects of research, surveillance and control of infectious diseases. **DIPNET** (contract 2005210) and **EU-Lab-DIPNET** have as main objectives the European harmonisation of laboratory methods, including molecular ones, for diphtheria diagnosis and surveillance. Other projects funded by ECDC have as objectives microbiological monitoring supporting activities (**ECDC/08/025/PC0035)**. The deliverables in this project are the country profile regarding communicable diseases, monthly informative bulletins, nation wide dissemination of documents, guides and publications issued by the ECDC. We also participate in a study started by the ECDC for the set-up of an integrated platform in support of European Vaccine Epidemiology. Laboratory proficiency is annually evaluated by EQA coordinated by ECDC.

Projects funded by structural funds

National Institute of Research and Development for Microbiology and Immunology „Cantacuzino" (NIRDMI "Cantacuzino") is currently implementing a strategic infrastructure project entitled "**Development of research infrastructure in microbiology, immunology and biotechnology for an increase capacity in investigating diseases with major impact on public health**" co-financed from the European Regional Development Fund (ERDF) as part of the EU Structural Funds (Sectoral Operational Programme "Increasing Economic Competitiveness"), and from national budget in order to build a sustainable research infrastructure and enhance personnel performance. Researchers from our institute are also participating in two **POSDRU** projects aimed at training of personnel in the fields

of immunology and biodiversity. The European project „Microwaves ecofriendly alternative for a safe treatment of medical waste" (**LIFE10 ENV/RO/000731**) is a project for innovation in bio safety domain.

Other external contractors

Studies on genetic subtypes and antiretroviral drug resistance of HIV-1 in Romania are the target of a project (**NAMRU)** funded by the US Defence Department. A NATO funded project aimed at the development of a novel immunoassay for the very early detection of biothreatening bacterial infections (**SfP Project No. 982838).** A highly specific method based on detection of alpha protimozine in blood will be validated for early detection.

Biopharmaceuticals is one of the fastest developing high technology industry in the world. Therefore one of the priorities of the institute was to improve development capacities in the field. In the absence of a strong specific industry in Romania the development was focused on our own products. Also the high amount of resources needed was obtained by external grants. WHO influenza vaccine production capacity building grant (Letter of Agreement SPHQ09-LDA-86 - $ 1.200.00) was dedicated not only for technology, but also for developing research infrastructure. A grant for the development of an adjuvanted influenza vaccine together with Infectious Disease Research Institute, Columbia Street, Suite 400 Seattle, WA 98103 (F16SUB-2010 - $474,408) was a subsidiary of the main grant 1 IDSEP100008-01-00 1124 funded by de ASPR/BARDA/AMS, „Development and Sustainable Manufacturing of Adjuvanted Pandemic Influenza Vaccines in Developing Countries". The scientific objectives consist in successful transfer of adjuvant production and characterization technology from IDRI to Cantacuzino to facilitate availability of a pandemic H5N1 influenza vaccine that demonstrates efficacy, antigen sparing capacity, and safety in preclinical animal models.

Main achievements resulting from research activity

Publications in journals with non-zero relevance score

A number of 105 articles published in international journals having a non-zero article influence score, totalling a score of 192.35932, resulted from national and international projects implemented by researchers of our institute. As a consequence of the collaboration within a consortium the most of the articles were written by a multidisciplinary, international co-workers team.

Other relevant publications

An important number of articles (127) were published in journals indexed for ISI citations but without article influence score and in journals indexed in international data banks such as Medline, PubMed, Chemical Abstracts, Directory of Open Access Journals, Open J-Gate, Genamics Journal Seek, CAB Abstracts, Media Finder®-Standard Periodical Directory, Mosby Nursing Consult/ Mosby Index, Scopus, Index Copernicus etc. The abstracts/integral texts for a total of 38 presentations to the prestigious scientific manifestations in the field of our activity were published in proceedings or in journals ISI indexed. The scientific contribution of our institute researchers was reflected in 45 books, handbooks and chapters in collective volumes.

As a result of the project "Combating flu in a combined action between the industry and the public sector in order to secure adequate and fast intervention in Europe" Flu Secure, a DG-SANCO project, a series of control reagents were developed together with the National Institute of Biological Standards and Control (UK) for the pandemic vaccine candidates. Also after the technology transfer of

the oil in water adjuvants methods for adjuvanted influenza vaccine preparation and control were developed and will be tested next year. This is improved with the benefits from the developments in FastVAC project with technology transfer and improving detergent splitting technology. These achievements represented also a major change in the development concepts traditionally based on own resources towards a modern collaborative integrated research.

Also a big achievement is the creation of a dedicated vaccine development infrastructure with pilot areas, modern equipment, animal experiment facilities. For instance in the In WHO influenza vaccine production capacity building grant all the phases of a modern product were present from the optimisation of production technology and controls to non-clinical and clinical trials. The pilot area was made from WHO influenza vaccine production capacity building grant extended with the adjutant equipment from IDRI/BARDA development grant. Also setting-up of a GLP laboratory for animal experiments, currently in progress was the result of national capacity building projects, WHO influenza vaccine production capacity building grant, and will benifit in its development from IDRI/BARDA development grant.

NIRDMIC is the location of the National Reference Laboratories for infectious diseases excepting HIV and tuberculosis. Besides research activities these laboratories are offering services for clinical and public health microbiology laboratories all over the country. They are involved in identification, confirmation and typing of microbial strains isolated from different human, animal or environmental sources. Also, they are involved in the National Programs for surveillance and control of communicable diseases and lay down the microbiological reports to be referred in TESSY data bank to ECDC. The laboratories are organizing courses and training for microbiologists and resident physicians who are performing their preparative programs for microbiology in the Reference laboratories.

Two CEEX projects M4 were finalized by implementation of accredited laboratories for „testing the efficiency of drugs recommended in the therapy of chronic and acute inflammatory diseases" and "for microbiological control of medical devices and for in vitro diagnostic according to the approved standards". For the successful completion of objectives in the BARDA project the set-up of a GLP laboratory for animal experiments is in progress.

As members in directory board of the Romanian Society of Immunology and Romanian Society of Microbiology we are participating as members in scientific committee to organize the Annual Conferences of these societies.

Since 1928 Institute is editing a peer-reviewed quarterly journal indexed in Medline/Pubmed, the "Romanian Archives of Microbiology and Immunology", (Print ISSN: 1222-3891, OCLC: 25545262).

T1_Applied Microbial Genomics

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Mission, objectives, research interest

The team has as main objective the development of newer improved tools for the etiological diagnosis and molecular surveillance of communicable diseases. In order to implement these methods in practice, Molecular Microbiology and Epidemilogy Team is developing **research collaborative projects** and is conducting **training activities** for students, master students, residents, PhD students and specialists from clinical and public health laboratories.

General objectives of our activity are consisting in:

- Strengthening the professional and institutional capacity for an effective participation to national and international programmes for research, control and surveillance of infectious diseases;
- Developing/optimization of the new molecular techniques used in diagnosis and sub-

typing of etiologic agents of infectious diseases in order to trace the circulation of pathogens over the country and across the border;

- Harmonization of the microbiological methodologies for a competitive participation in international projects of research, surveillance and control of infectious diseases;
- Promotion of the national and international co-operation in the field of the infectious diseases.

Specific objectives

- Molecular studies on virulence of pathogen, commensally and/or opportunistic bacteria involved in human infectious diseases;
- Studies on molecular and genetic mechanisms of antibiotic resistance;
- Improvement of laboratory methods used for diagnosis, epidemiology and treatment of communicable diseases using molecular techniques;
- Molecular diagnosis and characterization of non-cultivable micro organisms;
- Evaluation of the impact of microbial variability on diagnostic methods and microbial pathogenesis.

Research activity

The activity developed by the team between 2007 and 2011 focused on creation good conditions for collaboration within European frame research projects. The research projects aimed the improving the laboratory capacity to diagnose the communicable diseases occurred in community/hospital acquired infections, emergent and re-emergent infectious diseases and diseases due to commensally bacteria acquiring pathogenic factors by genetic events. The molecular methods such as RFLP methods (ribotyping, PFGE typing), PCR-based methods and sequencing are used for bacterial and viral identification and genotyping in order to establish the clonally spreading of strains and the phylogenetic relationship and evolution of pathogens.

The main themes of research could be summarized as follows:

1. Detection of the pathogenicity properties of commensally/opportunistic bacteria involved in human infectious diseases.

Classical and PCR-based methods were used in order to detect the vaginal carriers of pathogenic bacteria in females in order to appreciate the infection risk for UTI (**IDEI 721/2008** project coordinated by Codruta-Romanita USEIN), or for monitoring the emergent bacteria in different types of infections (**CEEX 143** scientific responsible Maria DAMIAN). Incidence of different bacteria in dental pathology

was studied in order to evaluate the antimicrobial activity of some lactoferrine compounds which could be used in treatment of oral affections (**IDEI 254/2007** project coordinated by Maria DAMIAN).

Use of molecular methods to detect the aetiology of diarrheic syndrome in children is the main objective of the projects **CEEX 47** coordinated by Maria DAMIAN).

2. Emergence of antimicrobial resistance.

RCR-based methods were used to detect the presence of *bla*-genes and RFLP and/or sequencing were applied on amplification products in order to identify the SHV, TEM and CTX-M type of enzymes coded by these genes. The resistance to quinolones was investigated targeting the *gyr* and *qnr* genes by PCR and by amplicon's sequencing. PFGE was used as molecular typing method to assess the clonal dispersion of resistant strains (**42-094** project, scietific responsible Maria DAMIAN; **NUCLEUS/PN 06150104** coordinated by Monica STRAUT).

Integrons, involved in antimicrobial resistance of gram negative bacilli and especially detected in *Pseudomonas aeruginosa* strains, *mec* cassette identified in meticillin resistantes isolates of *Staphylococcus aureus* and molecular markers for antiviral resistance in HCV infections are focused in the study developed within the NUCLEU project **PN 09 22 01 01** coordinated by Monica STRAUT).

3. Use of molecular methods for tracing the infections

Molecular methods are used for diagnosis and molecular typing of gram negative bacilli involved in food-borne infections (**42-106** project coordinated by Maria DAMIAN) and associated with immunological methods they are component of a functional model for surveillance and characterization of aetiological agents of endocarditis (**42-119** project coordinated by Monica STRAUT).

4. Improve the laboratory methods used for diagnosis, epidemiology and treatment of infectious diseases using molecular techniques

The correlation of the genotypic and phenotypic profile of *Helicobacter pylori* isolates in order to improve the short and long term disease evolution (**42-155** project scientific responsible Maria DAMIAN) was the main subject of a collaboration with a children hospital in Bucharest.

5. Use the molecular methods to detect human genetic disorders

Use DNA sequencing in order to characterize the mechanisms involved in Factor V activity and the study of relationship between factor V and clinical symptomathology in trombophilia is the study subject of project **42-099**, scientific responsible Maria DAMIAN).

6. New genetic markers used for microbial identification

Use of PCR-sequencing method targeting genes coding for rRNA 16S was the main objectif of a NUCLEUS project coordinated by Monica STRAUT **(PN 06 15 01 02).**

7. An important objectif of the team was the involvement in international research projects in order to improuve the activity and to increase the international competivness

As competent body for the laboratory surveillance of communicable diseases the institute is participating to the surveillance programmes established by ESDC. We are participating as national contact point for laboratory surveillance of infections due to Salmonella (Maria DAMIAN), E. coli (Codruta-Romanita USEIN) and diphtheria (Maria DAMIAN). In such a position we are participating to the external quality controls for the above microorganisms and in collaborative projects **(DIPNET contract 2005210 and EU-Lab-DIPNET).** The aim of the projects is the european armonisation of the laboratory methods, our team participating for classical microbiological methods as well as for molecular ones.

As member of International Network of Pasteur Institutes we are participating in collaborative research projects with other Pasteur Institutes. The **ACIP A 01-2009** is a project aiming to armonise at the Pasteur Network level the methoods for diagnostic and typing of *Corynebacterium diphtheriae* strains. A new molcular typing method (MLST) was implemented in our laboratory.

8. New project proposals

Following our main objectives, this year we participated in new proposals for the future activity.

A new project was proposed within the frame of the National Research Programme, Parteneriatsheep Program **PCCA 2011** (proposal title Improved algorithm to enhance Detection Capacity for Bioterrorism Response and Natural unusual outbreaks containment **Code PN-II-PT-PCCA-2011-3.2**-1506, proposed by Maria DAMIAN)
In order to improve the laboratory detection of Bordetella infections, a project in public parteneriat was initiated in 2011 aiming to implemet the real-time PCR technique as diagnostic method in pertussis infections (coordinated by Maria DAMIAN)
We participated to a proposal **FP7-HEALTH-2012-INNOVATION-2**, coordinated by Mr. Gilbert SkorskiPHYLOGENE (304858-1Proposal acronym: NOSOFIGHTP, proposal title „Direct sample characterization (identification, virulence and antimicrobialresistance mechanisms) of bacterial species involved in Healthcare Associated Infections, with faster turnaround time").

Starting this yaer we are participating also in a **COST action** reference **oc-2011-1-9598** for a COST new Action „Network for diagnostics of unknown pathogens (NUPATH)" cooedinated by Prof. Claude P. MULLER.

ISI publications

- Shona Neal, Bernard Bell, Kim Ekelund, Brigitta Henriques-Narmark, Aftab Jasir, Dwight Johnson, Edward kaplan, Marguerite Lovgren, Ralf rene Renert, **Members of Strep-EURO Study Group** and International Streptococcus reference Laboratories, Androulla Efstratiou. International Qualiry Assurance Study for Characterization of Streptococcus pyogenes. *J Clin Microbiol* (2007), 45, 4:1175-1179

- Cecile Evrin, **Monica Straut,** Neli Slovova-Azmanova, Nadia Bucurenci, Adrian Onu, Liliane Assairi, Mihaela Ionescu, Nicolae Polibroda, Octavian Barzu, Anne-Marie Gilles. Regulatory

Mechanisms Differ in UMP Kinases from Gram-negative and gram-positive Bacteria. *J. Biol Chem.* (2007), 282, 10:7242-7253

- C. Chilom, D. Gazdaru, N. Bucurenci, **M. Straut,** C. Geanta, A. Popescu – „ Fluorescent study of the interaction between *Staphylococcus aureus* UMP kinase and UTP" – *J. of Optoelectronics and Advanced Materials* 2007, 9 (8): 2585-2588

- Bogdan Luca-Harari, **Monica Straut**, Silvia Cretoiu, Maria Surdeanu, Vasilica Ungureanu, Mark van der Linden, Aftab Jasir. Molecular characterization of invasive and non-invasive *Streptococcus pyogenes* isolates from Romania. J *Medical Microbiol,* 2008, 57: 1354-1363

- Theresa Lamagni, **the Strep-EURO Study Group (Monica Straut)** and Aftab Jasir. Epidemiology of Severe *Streptococcus pyogenes* Disease in Europe. *J Clin Microbiol,* 2008, 46 (7): 2359-2367;

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- Martinez-Jéhanne V, du Merle L, Bernier-Fébreau C, Usein C, Gassama-Sow A, Wane AA, Gouali M, 7. Damian M, Aïdara-Kane A, Germani Y, Fontanet A, Coddeville B, Guérardel Y, Le Bouguénec C. , Role of deoxyribose catabolism in colonization of the murine intestine by pathogenic Escherichia coli strains. Infect Immun. 2009, 77(4):1442-50. (Impact factor: 3.987)

- Bogdan Luca-Harari, Jessica Darenberg, Shona Neal, Tuula Siljander, Lenka Strakova, Asha Tanna, Roberta creti, Kim Ekelund, Maria Koliou, Panayotis Tassios, Mark van der Linden, **Monica Straut,** Jaana Vuopio-Varkila, Anne Bouvet, Androulla Efstratiou, Claes Schalen, Birgitta Henriques-Normark, the Strep-Euro study group and Aftab Jasir. Clinical and Microbiological Characteristics of Severe Streptococcus pyogenes Disease in Europe. *J Clin Microbiol,* 2009, 21 January (*Impact factor 3.945*).

- **Codruta-Romanita Usein,** Dorina Tatu-Chitoiu , Simona Ciontea, **Maria Condei, Maria Damian.** Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. *Japanese Journal of Infectious Diseases* 62 (4): 289-293, 2009 (*impact factor 1.222).*

- Földes Annamaria, Oprea M., Bilca D.-V., Farkas L. A., Szekely Edit, **Străuţ Monica**, Dobreanu Minodora – „Posterior mitral valve endocarditis accompanied by bacteraemia with *Granulicatella adiacens*. Prezentare de caz." – Roum Rev Lab Med. 2009, 16 (3): 49-57. *(ISI, no impact factor))*

- Iuliana Babiuc, Mihaela Păuna, Mădălina Adriana Maliţa,Simona Georgiana Ariton, **Maria Damian**, Vasilica Ungureanu, Anca Petrini. Correct complete denture rehabilitation, a chance for recovering abused tissues. *Romanian Journal of Morphology and Embryology* 2009, 50(4):707–712. *(ISI, no impact factor)*

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36. Monica Straut, **Sorin Dinu**, Silvia Cretoiu, Brindusa Lixandru, **Maria Surdeanu** – Poster **P775:** "Molecular characterization of genetic structures surrounding *bla*$_{VIM-2}$ gene identified in *Pseudomonas aeruginosa* clinical isolates from Romania" – 20th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) – Viena, 10-13 aprilie 2010

37. **Monica Straut**, Silvia Cretoiu, Vasilica Ungureanu, Anca Petrini, Raluca Georgescu, Laura Grigore, **Codruta-Romanita Usein** – Poster **P1003:** "Distribution of serotypes, antimicrobial resistance and virulence-related genes among Group B streptococci of vaginal origin isolated in Romania" – 20th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) – Viena, 10-13 aprilie 2010

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The continuous education of young researcher is the characteristic of the scientific policy of the team.

Cretoiu Silvia, Andi-Marian Palade, Baltoiu Madalina and Dinu Sorin graduated the Master school in Microbiology and Biotehnology at the University of Bucharest.

Cretoiu Silvia attended a master course within the "Erasmus Program" funded by the Euopean Commission in the "Democritus" University, Orestiada, Greece, (1 Febr – 1 May 2007), and an Workshop on "Genetic methods used in microbial ecology studies" in the same University (April 2007).

Oprea Mihaela and **Andi Marian Palade** started their PhD student program in Oct 2007**.**

Dinu Sorin followed a training stage in Pasteur Institute Paris in 2011 (March and June)

Mihaela Oprea, Monica Straut, Irina Codita are members of SeqNet and they are participating in workshops and meeting of the network.

Maria DAMIAN, as contact point for laboratory surveillance of infections due to *Salmonella* and *Corynebacterium* is participating in annual meetings organized by ECDC. Workshop *"Intersectoral collaboration for detecting, surveillance and response to foodborne diseases*", organized by ECDC Regional OMS Office and Salm-Surv, (25-28 May, Zegrze, Poland is one of the important workshops attended.

Codruta-Romanita Usein as contact point for laboratory surveillance of infections due to the pathogenic strains of E. coli is attending meetings and workshops organized bt ECDC on this subject.

The team members are referee for Rom. Arch.Microbiol.Immunol (indexat PubMed) and

Bacteriol. Virusol. Parazitol. Epidemiol. Maria Damian is member of board of The Open Epidemiology Journal (Chemical Abstracts, Directory of Open Access Journals, Open J-Gate, Genamics JournalSeek, CAB Abstracts, MediaFinder®-Standard Periodical Directory, Mosby Nursing Consult/ Mosby Index, Scopus.

T2_Viral Respiratory Infections

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Mission, objectives, research interest

Laboratory departments:

Laboratory is structured in two departments: National Influenza Centre (NIC) and other viral respiratory infections and National Reference Centre Measles / Rubella, both units being components of National Surveillance Programes.
The main objectives of the laboratory are:

1. monitoring of influenza viruses circulation in Romania including avian influenza in humans, occurrences of new antigenic and genetic strains and antiviral sensitivity.

2. monitoring of immunogenicity and effectiveness of influenza vaccine produced by "Cantacuzino" Institute and new directions: development of cell-based vaccines for influenza viruses

3. monitoring of other respiratory viruses (Syncitial Respiratory Virus, Coronavirus, human metapneumovirus, parainfluenza virus, adenovirus etc.) involved in severe acute respiratory infections in patients (adults and children) from ICU (intensive care units) in Romania.

4. monitoring of measles and rubella virus circulating in Romania, genotyping of autochthon or imported viruses and prevention of congenital rubella syndrom.

NIC is nominated by the MoH and recognized by WHO as national reference laboratory for influenza since 1969, and accredited by RENAR body. Also, NIC was nominated by WHO as regional influenza centre in 2008. NIC -NIMRD Cantacuzino together with National Institute for Public Health coordinates national influenza surveillance system: ILI (Influenza like Illness) and SARI (Severe Acute Respiratory Infections). CNG-NIMRD Cantacuzino ensures the participation of Romania to the European system (Tessy ECDC Euroflu) and the international system (GISN-FluNet WHO) influenza surveillance. CNG-NIMRD Cantacuzino organizes national and international trainings for specialists in virological and serological diagnosis of influenza. CNG-NIMRD Cantacuzino participates in the development of protocols and methodologies for the laboratory diagnosis of influenza and other respiratory viral infections. Cantacuzino NIMRD CNG participates in developing, updating and implementation of national intervention in the pandemic. NIC insures quality control and trainings for sub-national influenza laboratories (Constanta, Bucuresti, Iasi, Timisoara and Cluj)

National Reference Center Rubella / Measles was nominated by MoH in 1999 and annually certified by WHO since 2005 and accredited by RENAR body.

The main objectives are virological and serological surveillance for measles, rubella and congenital rubella syndrome in Romania.

The goal is:

to investigate the measles and rubella viral transmission in sporadic cases and outbreaks by detecting specific antibodies in serum by ELISA;

virus isolation, molecular detection and molecular characterization by sequencing of measles and rubella virus.

Research activity:

Research projects:

1 - National

• *EVIRNET*: circulation and emerging multidisciplinary research zoonotic viruses, *agents of avian influenza*, of some hemorrhagic fevers and infections of neuroinvazive, an innovative concept for monitoring the multiple interfaces: health human / animal health / wildlife / environmental Dr. Cornelia Ceianu

• *AVRI: Evaluation of the molecular profile of influenza viruses for antiviral resistance and its clinical relevance*. The project was realized with a good colaboration with "M. Bals " Institute and " V. Babes" Department of Infectious and Tropical Diseases.

The project facilitated the *improvement of influenza virus by real-time PCR diagnostic, of RSV and hMPV RT-PCR detection by clasic RT-PCR*.

One of the major directions in research was to *evaluate the sensitivity of the influenza virus isolates the antiviral drugs - antineuraminidase by phenotyping (NA-STAR) and amantadine by sequencing (point mutations).*

EVAGRIP: the main objective was to evaluate the *immunogenicity of avian influenza virus (adjuvanted or not with allum) in preclinical studies and immunogenicity of seasonal influenza viruses administered in different ways in clinical studies*.

• *TERASER*: The main objective was to obtain *polyclonal antibodies against a target antigen in animals.* Our lab were charged with the influenza virus purification (PR8) in order to be used as an immunogenic agent of animals and test of humoral response by haemagglutinin inhibition assay and microneutralisation test.

• *CELLVACC*: cellular technologies for vaccine production. The main objective was to develop a *model for preparation of viral vaccines on cell lines grown in synthetic medium*, using modern technology to replace those who use eggs or chicken embryos primary culture (chicken embryonic

fibroblasts).

• *RESP-E 137*: Testing, evaluation and improvement of strategies for the prevention of respiratory infectious diseases in elderly population

• *Development of cell-based vaccines for influenza viruses:* - Interest in obtaining cell vaccines has been extended to influenza vaccine. Of great interest worldwide are disposable technologies that increase efficiency and safety manufacturer. The aims of project was the development of this technology by:
- Optimization of conditions for obtaining cell mass in type disposable bioreactor "wave";
- Optimizing production of influenza virus as a "wave" in small volumes, the development of work procedures and activities involved in the process control;
- Development of influenza virus production as a "wave" in large volumes (scale-up).
- Use of cell growth media and the development of alternative media alternative.

• *MIRVI: Molecular investigations of the acute respiratory infections brought on by non-flu viruses, assessing the involvement in the innate and child's pathology.*

The aim of the project was to investigate the non-flu respiratory infections (caused by the Syncitial Respiratory Virus – VRS, Human Meta Pneumo Virus – hMPV, Para flu Viruses 1, 2, 3, Adenoviruses and Corona viruses OC 43 and 229E) using molecular assays to evaluate the etiological agents and to evaluate the host reaction to them, using both clinical data and molecular laboratory tests, for the etiologic diagnostic, also to analyze the inflammatory soluble mediators (sera citokines/chemokines).

The project main objectives are: - to evaluate the infections, using both modern techniques of pathogen detection (RT-PCR/real time RT-PCR), and those assays that canalizes the host reactions using the inflammatory soluble mediators patterns (Multiplex technology – xMAP, applied on the LUMINEX 100IS platform);

- assessing the clinical relevance of the molecular profile,

- to estimate the involvement in the diagnostic strategy, management and control of respiratory infections of the child.

• *CTVIAS 164* - Control of vertical transmission of infections associated with pregnancy by testing pregnant women and newborns. The main purpose of our lab was to monitor the role of infection with *rubella virus and parvovirus B19* of pregnant women. New techniques were implemented in our lab, such as *rubella avidity test in the diagnosis*, but also *measles virus detection and genotyping* due to the differential diagnostic of measles and rubella infections.

• Strengthening and expanding microbiological surveillance in vaccine preventable diseases: measles, rubella, mumps, polio, diphtheria

Objectives:

- *Enhancement of molecular surveillance of measles virus, the genetic differentiation of the wild virus vaccine, phylogenetic analysis*;
- *Enhancement for rubella and CRS diagnosis by RT-PCR and genotyping introduction*, knowing that the serological diagnosis is not always appropriate or sufficient;
- *Establishing a core of mumps virus detection to monitor its movement in the future and diagnosis of disease or complications*.

2 - International projects

• *FLUSECURE*: "Combating flu in the combined action between the industry and the public sector in order to secure and fast Adequate Intervention in Europe" 2006 - 2008; objectives were: *evaluation of immunogenicity of H5N1 influenza virus in mice and antibody persistence*, other activities have been *collaboration between IC and NIBSC for filling, lyophilization and calibration of reference reagents to make a bank of reagents and the study of vaccine strains in cell culture propagation*.

• *Rivers*: "Resistance of influenza viruses in Environmental Reservoirs and Systems" The main purpose is to *study the impact of water treatment on survival H5N1 virus, the objective is to find the best combination of agents with the conditions of pH, salinity , temperature acting on virus survival in water*.

• *ConFluTech: diagnostic technology transfer of emerging viruses* - training for specialists from three countries held in our laboratory.

• *IMOVE: t*he objective is to *monitor the effectiveness of pandemic/seasonal influenza vaccine in EU / EEA member states*, during the following season: 2008-2009, 2009-2010, 2010-2011, 2011-2012

• *European programme ECDC and WHO programme for:*

8. Pandemic Preparedness activities in the South-Eastern European Countries: 14400 $ - 2009 (WHO)
9. Pandemic Preparedness activities in the South-Eastern European Countries: 33500 $ - 2009-2010 (WHO)
10. Pandemic Preparedness activities in the South-Eastern European Countries: 45000 $ - 2010-2011 (WHO).

• *Grant WHO*: WHO influenza vaccine production capacity building grant Grant Agreement: SPHQ-LOA-86–Dr.Onu

• *BARDA* – The goal of this project is to successfully transfer adjuvant production and characterization technology from IDRI to the Cantacuzino Institute to facilitate availability of pandemic influenza vaccines that demonstrate protective efficacy, antigen sparing capacity, and safety in preclinical animal models. This goal will be achieved by employing IDRI's extensive adjuvant production and technology transfer experience in combination with the influenza antigen production and preclinical animal model expertise of the Cantacuzino Institute.

• *FastVacc* - The Cantacuzino Institute is currently producing a split influenza seasonal vaccine. Regarding the fact that the splitting agent used, ether, has high risk of explosion, on request of WHO, the Cantacuzino Institute wishes to change over to detergent as splitting agent. Initial experiments were started in consultation with the National Institute for Public Health and Environment (RIVM), Bilthoven, The Netherlands, to compare the current process with the desired process.

The results of these experiments show that the implementation of new splitting technology as well as the inactivation technology are suitable to be introduced as steps in actual technological process. Preliminary experiments showed that those steeps produced material with promising characteristics in term of stability while they yield and productivity are comparable and are in the expected values. We are currently performing experiment aimed to finalize the characterization of the products.

• *Proposal:*

- Study of pathogenicity and virulence markers of influenza virus in adults and children with severe acute respiratory infection, correlated with immune host response and clinical evolution (VIRPATHO)
- Evaluation of molecular interactions in severe co-infections of respiratory tract, with influenza and non-influenza viruses and S.pneumoniae, S.aureus, H.influenzae, Mycoplasma pneumoniae and Legionella pneumophila, in order to improve the diagnosis and treatment strategies in Romania
- Study of viral agents – measles, mumps and rubella during the elimination phase of these diseases and prevention of Congenital Rubella Syndrome till 2015, target of WHO.

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Traning-uri:

- Interne:

- Cursuri pentru medicii de familie oganizate de MSP si JSI cu finantare USAID privind diagnosticul de laborator al gripei sezoniere/gripei aviare; Lect. V.Alexandrescu, D.Pitigoi:15 febr.,7 martie,20-21 aug.,23-24 aug.,17-18 sept.,18-19 sept.2007(Bucuresti,Ploiesti,Iasi,Constanta, Predeal)

- Cursuri pentru medicii specialisti oganizate de MSP si JSI cu finantare USAID privind medicina muncii; Lect. V.Alexandrescu;1 iunie-Sovata 2007

- Difficulties in quality assurance in laboratory medicine, INCDMI Cantacuzino, RoEQALM 01-02 October 2010 Bioch. Maria Elena Mihai, Biol Mihaela Lazar, Bioch. Carmen Marica

- GLP-Good Laboratory Practice Training Gourse, Liam OhAlmhain.B Sc. FICI Course Tutor and Trained Lead Assessor of Laboratory, INCDMI Cantacuzino, Bioch. Maria Elena Mihai, Biol Mihaela Lazar, Bioch. Carmen Marica, Biol. Alina Baetel 17-20 November 2010

- In cadrul proiectului "Strengthening overall management of influenza surveillance and laboratory capacities for influenza virus diagnosis (avian, AH1N1-2009, seasonal)" , derulat de INCDMI Cantacuzino si finantat de Ministerul Sanatatii din Republica Moldova si Banca Mondiala am organizat in perioada 7-11 December 2009, 14-18 December 2009, 9 – 23 iulie 2010, 3 cursuri cu titlul:"Aspecte ale diagnosticului si supravegherea gripei in sistem sentinela" pentru medici din Republica Moldova. Lectori Dr. Viorel Alexandrescu, E. Lupulescu, Sef de lucrari Dr. Daniela Pitigoi, Dr. Claudiu Sbarcea, Biol. Emil Ionita, Biol. Alina Baetel

- Internationale:

  - Training cu specialisti din medicina umana si veterinara din Bulgaria,Grecia si Turcia in cadrul proiectului ConFluTech 10-14 dec. 2007-Tema diagnosticul molecular al infectiei gripale;coordonatori;E.Lupulescu;V.Alexandrescu

  - Training- ESWI ,ERASMUS Medical Center Rotterdam (Olanda): Diagnosticul virologic si serologic al gripei;1-7 iunie 2007 participant Dr.C.Sbarcea

  - IATA Training workshop(OMS),Sankt Petersburg, 9-11 Iulie 2007:Dr.C.Sbarcea

  - Training on molecular detection and genotyping of measles virus at the Sub-Regional Laboratory of WHO EURO, "Robert Koch Institute", 1- 11 June, 2008, Gheorghe Necula

  - Training in Influenza Diagnostics using molecular biology techniques, 18-22 August 2008, at National Influenza Centre, SSI, Copenhagen, Denmark, Emilia Lupulescu, Maria Elena Mihai

  - ConFluTech Workshop on AIV Sequecing, Phylogeny and Bioinformatics, 26-29 Mai, 2009, Uppsala, Suedia: Dr. Emilia Lupulescu, Biol. Alina Elena Baetel

  - Influenza Practical Training Course, Erasmus Medical Center Rotterdam, 8-11 Iulie 2009, Olanda: Biol. Alina Elena Baetel

- *"Laboratory Biosafety Level 3 Training Program, University of Texas, Galveston, USA July 19-23* 2010- Dr. Emilia Lupulescu

- *CNRL Influenza Surveillance Training Course, HPA London, June 7-11,* 2010*,* biochimist. Carmen Maria Cherciu

- Tehnical Workshop on basic molecular biological techinques, Research Center Borstel, Germania, 01-12 November 2010, biolog Mihaela Lazar, biochimist Carmen Maria Cherciu

- Community Network of Reference Laboratories for Human Influenza în Europe (CNRL), "Influenza Sequencing and Bioinformatics Training course", HPA, London, UK. 29 October 2010- 03 November 2010, G. Necula

- Laboratory Quality Management System Training Belgrade, Serbia, National Institut of Publich Health 14-17 December 2010. Biol Alina Bãetel

- Training on transport of infectious substances, Ljubljana, Slovenia, 9-10 June 2011 Biol Alina Bãetel

- Influenza serology training course, CDC Atlanta, USA, 11-22 July 2011 Biol Alina Bãetel

- "CNRL Antiviral Training Course 4-8th July 2011" HPA (Health Protection Agency), Londra Bioch. Maria Elena Mihai

- Workshop Classical and molecular veterinary virology – 28.11-09.12.2011, Viena – Veterinary Medicine University – Gheorghe Necula

Perfecţionarea resursei umane (*cursuri, diplome de master, doctorat*):

- Diploma master „Neurobiologie", Facultatea de Biologie, Universitatea Bucuresti, iulie 2008: Biolog G.Necula

- Diploma master „Genetica, Microbiologie si Biotehnologie", Facultatea de Biologie, Universitatea Bucuresti, iulie 2009: Biol. Alina Elena Baetel

- Diploma master „Neurobiologie", Facultatea de Biologie, Universitatea Bucuresti, iulie 2009: Biochimist Carmen Maria Marica

- Diploma doctorat in biologie, Facultatea de Biologie, Universitatea Bucuresti, 2011, Biolog G.Necula

Organizare de: workshopuri:

ACTUALITATI IN SUPRAVEGHEREA SI DIAGNOSTICUL DE LABORATOR AL GRIPEI AVIARE" CONFLUTECH - Bucuresti - INCDMI –CANTACUZINO 26-27 – Septembrie 2011

T3_Vaccine preventable diseases

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Ilie Ana Maria – med techn (0.25)

Ionita Vasilica – laborant (0.25)

Alina Penciu, med techn (0.25)

Adriana Metcan, med techn (0.25)

Mariana Popescu, laborant (0.5)

Elisabeta Tomita, laborant (0.25)

Mission, objectives, research interest

The team consists from staff working in the laboratory of bacterial respiratory diseases and viral enteric diseases and they are representing the National Reference Centre for Enteroviruses and, from 1995, part of the Global Network of WHO laboratories for polio, annually accredited and National Reference Centre for Bacterial Respiratory Diseases which is dealing with diphtheria, pertussis, Haemophillus, Meningococcus and Stretococcus infections.

The main objectives of the team can be summarized as follows:

Studies regarding the circulation of polioviruses and non-polio enteroviruses in Romania, as a part of the National Program for Poliomyelitis Eradication

Molecular approach of identification and genotyping of enteroviruses and studies regarding the infection tracing within an international team organized at the level of International Network of Pasteur Institutes.

Studies on improving the quality of laboratory surveillance of invasive/non-invasive streptococcal diseases;

Improving the laboratory diagnosis of pertussis diseas by implementing the molecular methods of analysis

Armonization of the methods used for detection and characterisation of toxigenic Corynebacteria

Participation in a pilot study for monitoring the bacterial meningitis in children under 5 years, in collaboration with CNPCBT

As Reference laboratories the staff members are involved in providing services for clinical and public health laboratories on vaccine preventable diseases.

Research activity

The research projects aimed the improving the laboratory capacity to diagnose the infectious diseases determined by vaccine preventable pathogens. The molecular methods such PCR-based methods and sequencing are used for bacterial identification and genotyping in order to improve the trasability of infections and establish the clonally spreading of strains and the phylogenetic relationship and evolution of pathogens.

As members of DIPNET we are working in an european network established at the ECDC level with the main aim to armonize the methods of diphtheria detection, and strain characterization. The **DIPNET contract 2005210** and **EU-Lab-DIPNET** project have as main objectives the laboratory surveillance of diphtheria diseases and implementation of the molecular methods in our laboratory for diagnosis and strains typing. Within the projects we are participating in annual external quality assays and **Cerasella Dragomirescu** participated in a training course on this subject (oct. 2011).

As part of the International Network of Pasteur Institutes we are participating in a ACIP project (**ACIP 01 2009)** aiming to armonize the laboratory methods for detection and strains characteruzation in diphtheria disease at the Pasteur Institute level. In colaboration with team of Molecular Microbiology and Epidemiology a new molecular typing method was experinced and introduced in the laboratory activity. Within this project **Anca Petrini** followed a training course for a mounth in Pasteur Institute Paris for laboratory diagnostic and surrveillance of diphtheria diseases (2010).

A Transverse Research Program **(PTR 276)** was developed in collaboration within Pasteur Institutes Network aiming to evaluate the recombination capacity of enteroviruses, in conditions of co-circulation, interactions et evolution of enteroviruses C and polioviruses in the different geographical regions (project coordinated by Francis DELPEYROUX Unité Biologie des Virus Entériques, Institut Pasteur Paris, **Anda Baicus** being the team leader for Romania.

National projects are focusing on:

1. Modern strategies for investigation and intervention in infectious diseases

Elaboration of a modern diagnostic and treatment algorithm for acute bacterial meningitis to allow a quick identification of the ethiologic agents, diagnostic orientation and treatment, by combining conventional methods with new methods and techniques characteristic to immunology and molecular biology, capable of highlighting disease pathogenie critical points, which could become alternative therapeutic targets (**Parteneriate 42-116/2008,** coordinated by Vasilica Ungureanu) and creation of a functional model for monitoring and characterization of etiologic agents involved in infectious bacterial endocarditis (collaboration within project **Parteneriate 42-119/2008)** are representing the research objectives of our team.

2. Studies regrding the pathogenesis and strains characterization of some streptococci involved in infections with diverse localization

Incidence of different streptococci species involved in mouth diseases, detection of genetic structures responsible for virulence and molecular characterization of strains as well as the studies on susceptibility of strains to lactoferines in order to use such biological molecules in infections treatment were focused in project **IDEAS 254** (colaboration). The **IDEAS 721 (**colaboration) focused the role of

vaginal carriage of some potentially pathogenic microorganisms in developing the future infections. The strains were izolated and their patogenic power was evaluated by molecular methods targeting genes coding for virulence.

3. Strengthening and expansion of capacity for microbiological surveillance in preventable diseases by vaccination: measles, rubella, mumps, polio, diphtheria"

A project **Nucleu** was developed within our team aiming to improve the capacity of rapid response to an „epidemiological alerte" by using rapide, sensible and sensitive methodes for diagnostic and strains characterization. Molecular methods targeting species specific gnetic regions for pathogen identification as well as for strains virulence/toxigenicity detection were tested in different experiments in order to be introduced in laboratory practice.

4. Genotyping of poliovirus strains isolated in Romania between 2007-2010 from AFP cases and healthy contacts as a main objective of the global polio eradication(IDEAS project) has as the main objectives the molecular characterization of poliovirus strains by the RT-PCR-RFLP assay and sequencage for an active surveillance of acute flaccid paralysis cases in Romania.

Publications in ISI journals

- Baicus C, Caraiola S, Rimbas M, Patrascu R, **Baicus A**; for Grupul de Studiu al Scaderii Ponderale Involuntare. Utility of Routine Hematological and Inflammation Parameters for the Diagnosis of Cancer in Involuntary Weight Loss. J Investig Med. **2011** Jun 25. [Epub ahead of print]
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T4_Vector-borne infections and medical entomology

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Mission, objectives, research interest

The mission of the research team is to scientifically fundament advanced diagnostic and surveillance of vector-borne viral, rickettsial and other bacterial infections, as well as of their vectors (mosquitoes, ticks, rodents) in this country

The general objective of our research activity is to provide reliable, scientifically valid tools to better prevent and control human disease caused by vector-borne agents in Romania and in Europe.

Specific objectives

- Optimization of advanced serological and molecular techniques used in diagnosis and typing of endemic and imported vector-borne pathogens;
- Multidisciplinary surveillance of vector-borne agents at multiple interfaces: human health, animal health, wildlife, and environment;
- Biology of vectors and eco-epidemiology of vector-borne diseases in the changing environment and changing climate;
- Development of tools for the management of pathogens with high biological risk.

Research activity

The research team on vector-borne agents and medical entomology combines specialized laboratory and field expertise, national excellence and extensive contacts with the specialized European research laboratories, in order to respond to the challenges presented by vector-borne

infectious diseases, with the objective to mitigate that risk. The research activities in the period 2007-2011 were in full agreement with FP7 research areas, with the national research programs and research needs in this priority domain, as expressed by European and global organizations such as European Centres for Disease Control and World Health Organization.

- Optimization of advanced serological and molecular techniques used in diagnosis and typing of endemic and imported vector-borne pathogens

The research lead to important achievements in modern diagnostic, in creating capacity for genotyping and molecular epidemiology investigations of the following agents: hantaviruses, West Nile virus, dengue viruses, yellow fever virus, Chikungunya virus, Legionella sp, Rickettsia conorii, Borrelia sp, Bartonella sp. The advanced tools have been validated by integrating laboratory work with patient clinical management in the projects no 42119/2008 (PN II / partnership: *Bacterial infectious endocarditis - development of a functional model for surveillance and characterizng of etiologis agents based on molecular and immunological methods.* Acronyme ENDOBACT, Director: Monica Straut) and CEEX 164/2006. (VIASAN program; *Control of vertical transmission of gravidity associated infections (CTVIAS), with research on a cost-efficient testing algorithm.* Participants : dr **D Badescu**, dr **A Cotar**. Budget 2007: 420 000 lei; 2008:790000 lei).

- Multidisciplinary surveillance of vector-borne agents at multiple interfaces: human health, animal health, wildlife, and environment;

This interdisciplinary direction of research was developed in the following projects:

- CEEX 86/2006. Viasan Program EVIRNET. Director: dr **C Ceianu**. Budget: 509000 LEI/ 2007year; 404500 LEI/2008 year. The EVIRNET project lead to outstanding new to science results and increased international visibility of the team and Coordinator organization: first records for the circulation in Romania of the following zoonotic viruses: Dobrava hantavirus, Lymphocytic choriomeningitis virus, Crimean-Congo hemorrhagic fever virus. The project also lead to increased international visibilility of the team and of INCDMI Cantacuzino. The scientists in this team participated in the FP7 call on vectors biology and vector-borne diseases (2010), as partners, in four project proposals, of which one has been selected for funding.

- CEEX 92 / 2006 (Biotech Program): *Transdisciplinary health/environment investigation on re-emergence of West Nile virus in the environmental changes in Romania, and a strategy to control infections* (Director **G Nicolescu**; period 2006-2008; budget: 2006 – 190000 LEI, 2007 – 450000 LEI, 2008 – 860000 LEI). The project conducted to better understanding of epidemiological processes leading to outbreaks of an important vector –borne agent.

- Project no 42-125/ 2008 (PN II/partnership). *National multidisciplinary strategies for early warning, monitoring and control of remerging mosquito borne diseases, in the European space*. Acronyme VECBOLEM, 2008-2011. director **V Ciulacu-Purcarea**. Budget: 2009 – 132000 LEI, 2010 – 195745 LEI, 2011 – 193186,85 LEI. This project lead to new methodology for risk assessment for malaria re-emergence, early warning, surveillance and control of West Nile human infections.

- Biology of vectors and eco-epidemiology of vector-borne diseases in the changing environment and changing climate;

A main direction for research was the eco-epidemiology of vector-borne diseases in the changing European environment which has been developed in the framework of FP6 and FP7 projects: EDEN (2005-2009) and EDENext (2011-2014).

- EDEN project (FP6, GOCE-CT-2003-010284; *Emerging Diseases in a changing European eNvironment*) aimed to identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change , which are influencing the spatial and temporal distribution of human pathogenic agents. The project developed and coordinated at the European level a set of generic methods, tools and skills such as predictive emergence and spread models, early warning , surveillance and monitoring tools and scenarios, decision support for intervention and public health policies at both EU and national or regional level. The research team participated in three subprojects: tick-borne diseases (coordinator dr **AF Vladimirescu**), West Nile virus (coordinator dr **G Nicolescu**), and malaria (coordinator dr **G Nicolescu**). The European Grant for the project was 454655 Euro, and for the analysed period it was, respectively: 2007 – 131744 Euro, 2008 – 43551 Euro, 2009 - 40510.8 Euro. New to science knowledge has been gathered for the circulation of West Nile virus, ticks as vectors of Lyme disease, and Anopheles mosquitoes potential vectors of malaria. Three doctoral theses were ellaborated in the framework of this project.

- *Biology and Control of Vector-Borne Infections in* Europe", acronyme EDENext, GA 261504. EDENexts is to be developed in four years (2011-2014). The total budget of this project is: 254160 euro, of which 190620 euro are represented by the European grant. Cofinancing for this project is supported by 147-1EU /2011 of National Authority for Research. The EDENext project addresses biological , ecological and epidemiological components of vector-borne diseases introduction, emergence and spread , and it proposes advanced tools for controlling them The INCDMI Cantacuzino team, coordinated by dr **C. Ceianu**, is involved in the studies of West Nile virus mechanism of maintenance in the European environment via vertical transmission and overwintering of mosquito vectors. Two doctoral fellows are preparing theses in the framework of this project.

- Development of tools for the management of pathogens with high biological risk.

We developed diagnostic capacity for modern serological and molecular diagnosis of Crimean – Congo hemorrhagic fever virus, Coxiella burnetti, Francisella tularensis. Researchers also participated in ArboZooNet project, an International Network for *Capacity Building for the Control of Emerging Viral Vector Borne Zoonotic Diseases*, a Coordination Action funded for 3 years by the European Union (EU) under FP7 that was launched in May 2008. International collaboration enabled study of circulation of high bio-risk pathogens borne by ticks.

Other relevant activities:

Scientists in the team are also performing studies related to the activity of reference laboratory centres at national level for arboviral infections and viral hemorrhagic fevers, for rickettsial diseases, Lyme borreliosis, legionellosis and Q fever, and for medical entomology. Staff participated also as partner in other project on entomology related topics: no 51-014/2007. (PNCDI Partnership projects). *Conservation of the genetic potential and of biodiversity of autochtonous sericiculture resources*. Acronyme COSERISTECH. Coord. Dr. **Al. Vladimirescu**; 2007-2010.

Other relevant activities of staff during this period were :

- participation (**C Ceianu, R Panculescu-Gatej**) in the project funded by the European centres for Disease Control (ECDC): Establishment of a Collaborative Network of European Laboratories for Outbreak Assistance and Support co-ordinated by the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD);

- ECDC focal point for Climate Change and participation to the project Climate and West Nile virus (**C Ceianu**);

- Participation as invited experts in ECDC meetings for West Nile virus, legionellosis, control of invasive mosquito species, and climate change **(C Ceianu, D Badescu**);

- Participation in elaborating ECDC guidelines for risk assessment for West Nile virus infections (**C Ceianu, G Nicolescu**);

- Participation in ellaboration of methodologies for surveillance and in laboratory-based surveillance in the framework of national programs targetting vector-borne diseases such as West Nile virus neuroinvasive infections, tick-borne encephalitis, Lyme disease, and legionellosis (**D Badescu, C Ceianu**, **A Vladimirescu, G Nicolescu**)

- **C Ceianu** was reviewer for Arch.Microbiol.Immunol (PubMed listed) and for Vector-Borne and Zoonotic Diseases., **A Vladimirescu** is member of the editorial board in “Romanian Journal of Genetics” ISSN 1841-2513, reviewer and to“Romanian Biotechnological Letters” ISSN 1224 – 5984; **Elena Coipan** is reviewer for Journal of Infectious Diseases and Immunity” ISSN 2141-2375, “Journal of Vector Ecology” ISSN 1081-1710, and “International Research Journal of Microbiology” ISSN 2141-5463.

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Human resources policy:

In the period 2007-2011 the young staff completed Masters’ degree in Biochemistry (**R. Panculescu-Gatej**), and Biostatistics (**Elena Claudia Coipan**) and enrolled into doctoral programs of the University of Bucharest (**Liviu Prioteasa, Elena Falcuta, Raluca Panculescu Gatej**). Two other young staff (**Ani Ioana Cotar**/ 2009 and **Elena Claudia Coipan**/ 2011) successfully defended their PhD theses and obtained their PhD degree in the University of Bucharest. **A Cotar, R Panculescu – Gatej** participated in training curses organized in our institut: *PCR training* (2007), *Molecular tracing of*

viral infections (2008), *Management of biological risk* (December 9-11, 2011; **A. Cotar**) or in courses organized at national level: *Genomycs/ Symp STAM 2010* (nov 2010; Bucharest, **A Vladimirescu**).

Also they participated to international training and courses: *6 th ESCMID Summer School* (Suceava, July 1-6, 2007/ **A Cotar**); *Entomological and mosquito control training* (EID Mediterranee , Montpellier France, June 16-23, 2007, **L Prioteasa**); *Molecular identification of mosquitoes* (Laboratory of Institut pour Recherche and Development/IRD, Montpellier, France , March 5-18, 2007, **E Falcuta**); *Training RLB for Borrelia burgdorferi gen*ospecies (Dec 7-12, 2008, Inst of Zoology, Université de Neuchâtel, Switzerland; **A Vladimirescu, E Coipan**); *Stratagene-University* (Nov 3-7, 2008, La Jolla, California, USA; **A Vladimirescu**); *West Nile Virus Diagnostic Techniques* (Istituto Zooprofilattico Sperimentale „G.Caporale", Teramo, Italy, April 27-29, 2010/ **R. Panculescu Gatej**), *Introduction to the Epidemiology Toolbox* (Nov 28- De 2, 2011) and *More of the Epidemiological Thinking and tool* (Nov 29-December 2), workshops organized by ConFlutech project and Freie University, Berlin (participant **R Panculesc-Gatej**), *Laboratory diagnostic fo Legionella infections* (London, HPA and ECDC , Nov 28- Dec 2, 2011; **A Cotar**), *Use of GIS in vectors surveillance* (Zoerstel, AviaGIS, Belgium, Dec.5-9, 2011; **L Prioteasa and E. Falcuta**)

Future directions

With the ecologic and climatic changes and its integration into global circulation of merchandise and of persons, Romania is at greater risk than ever from vector-borne diseases, and is also a port of entry for different vector-borne pathogens in the EU. The future work is directed towards interdisciplinary innovative research to predict, prevent and monitor vector-borne diseases, to the study of the driving actions of climate change and of biodiversity loss on vector-borne diseases emergence and spread, to the prevention of introduction of invasive vector species, to elaborating tools for risk assessment, for preparedness and response to events involving agents with high biological risk. In the present national competition for projects in partnership, the team participated with 3 projects as coordinator and 2 projects as partner; the project proposals are under evaluation.

T5 Blood and sexually transmitted diseases

Head of team: **Gabriela OPRISAN,** Sci, PhD, senior researcher (CS I), goprisan@cantacuzino.ro

Research staff:

Băncescu Adrian-Anton, MD, PhD, senior researcher (CS II)

Jidovu Andreea – Alexandra, Sci, scientific researcher

Dinu Sorin (1/2), Sci, scientific researcher

Other personnel:

Ionescu Dan, MD, PhD

Monica BALTEANU, MD, m.balteanu@cantacuzino.ro (pana in 2010)

Cristache Ionelia, med assist

Rusu Viorica, med assist

Cristea Marilena, med assist

Nitu Catalina, med assist

Geonea Madalina, med assist

Silvia Tudor, laboratory tehn

Mission, objectives, research interest

The team consists from two reference laboratories: Laboratory for sexually transmitted diseases, an acreditated laboratory involved in diagnosis and surveillance of such of diseases including HIV and reference laboratory for blood transmitted diseases with a special program for hepatitis.

The main objectives of team are:

Improve the professional and institutional capacity of team to participate in national and international programmes for research, control and surveillance of infectious diseases due to blood and sexually transmitted pathogens;

Development of new methods for an efficient diagnostic and surveillance of diseases, including the uncultivable, untypable and emergent viruses

Development and implementation of molecular methods for viruses genotyping and subtyping for tracing infections

The development of new PCR systems for the detection of mutations induced by antivirals and evaluation of resistance patterns

Continuous activity for methods armonisation in order to acomplish the role of reference laboratories by assessment of new technologies for recognition the pathogenicity and virulence factors and development of genetic markers useful for diagnostic.

We have expertise in design of primers, RT-PCR, sequencing, molecular analysis of sequences and phylogeny. We are interested in developing molecular methods for typing of viral strains as HCV, HBV, noroviruses, enteroviruses etc. The design of primers in the 3D region of enteroviruses (>90

serotypes) allowed the identification of natural circulating recombinants of two different serotypes of enteroviruses isolated from meningitis cases. We also evaluated "in-house" RT-PCR methods for genotyping HCV strains or resistance mutations and participated for identification of highly pathogenic avian influenza H5N1. This was the first evidence of H5N1 presence in fowls in Romania.

Research activity

- *Evaluation of the impact of the viral variability in the diagnosis and pathogenesis of viral infections*

This project evaluated by PCR-RFLP and PCR-sequencing genotype distribution of Hepatitis C virus in Romania, circulation and phylogenetic relationships between isolated strains, the role of mutation and variability of the viral protein core+1 encoded by an alternative open reading frame from core region in evolution of HCV strains and the impact on the pathogenesis (**CEEX 158/2006 project**). Both the genotyping and phylogenetic analysis' results from our study revealed that while subtype 1b is still dominant in the Romanian hepatitis C epidemic, infections with newly introduced genotypes (1a, 3 and 4) are emerging. Patients infected with the non-1b genotypes are younger than the rest and come from urban areas, where IDU is rapidly spreading. The genetic distances among the HCV 1a strains are very homogeneous and small, with a high sequence identity with other European strains, suggesting the recent entrance of this subtype in Romania from singular or limited sources of infection.

- *Variability of HCV and HBV connected to the Primary Liver Cancer Development*

The variability of HCV and HBV in sera compared with tumor and non-tumor tissue isolates was investigated by participation in the international project **ACIP 24/2007** - project coordinated by Pascal Pineau from Pasteur Institute- Paris. **Gabriela Oprisan** was the team leader for Romania. The role of viruses in carcinogenesis and corelation between genotypes and cancer evolution were the objectives of the project. We identified some tumor specific substitutions located in the N-terminal part of the HCV core protein. In silico analysis of the hypothetical F protein, encoded by a frame shift in core region, showed higher degree of sequence diversity than the core protein. As the results are encouraging, a new proposal was disscussed within the meeting of European Network of Pasteur Institute organised in Rome during October 2011.

3) *Studies regarding the emergence of resistance to antivirals*

The project **Nucleu PN 09 22 01 01** focused on developing molecular biology methods useful in surveillance of sensitivity to anti-virals, the cross-resistance and co-resistance. The detection and characterization of mechanisms involved in resistance to antivirals in HBV and HCV strains and the disemination of resistant strains was one of the objectives of the project. We developed an ARMS-PCR for detection of HCV resistance mutations to pegylated interferon and ribavirin and evolution to carcinoma. Recently, it has been reported that the impact of the **IL28B polymorphism** on response to therapy may be different in terms of race, geographical areas or HCV genotypes. In order to

identify human polymorphic genomic regions associated with treatment resistance to current treatment or the new antivirals and intra- and inter-patient HCV diversity by sequencing and ultra-deep sequencing we applied for a **new project** in the **frame of the National Research Programme PCCA 2011 in collaboration with a SME**.

In order to detect genotypes, to subtype them and to identify recombinants of hepatitis B virus, a PCR was optimised followed by sequencing in the genomic pre-S1, pre-S2 and S regions. The sequence analysis of HBV strains indicates the predominance of genotype D followed by genotype A. We identified therapy induced mutations (M204V/I) in the catalytic site of viral polymerase and the compensatory mutation L180M in patients previously treated with lamivudine.

4) *Molecular characterization of emergent and un-typable viruses*

The first evidence of noroviruses circulation in Romania, uncultivable viruses, responsible for cases of gastroenteritis, was proved by using RT PCR systems in nucleocapsid and polymerase genes. Genogroup specific primers detected strains belonging to genogroup I (GI) and to genogroup II strains (GII). Sequencing results revealed the close genetic relationship of the Genogroup II analysed viruses with a highly virulent norovirus strain circulating in Europe (GGII.4.Lordsdale). and a potential recombinant belonging to epidemic cluster GII.b (**CEEX 47 project**)

5) Molecular characterization of enteroviruses

The meningitis cases derived enteroviruses or untypable strains were analyzed by RT-PCR and sequencing in VP1-2A and 3D genomic region (home-made systems) for classification and phylogenetic relatedness evaluation. Among untypable, two genotypes of Echovirus 33 were identified having originated from epidemic strains circulating in the world (New Zealand).

As director of Training Center, Gabriela Oprisan is involved in courses organizing the following training courses were organized :

- Sequencing used in the diagnostic and epidemiology of infectious diseases – 24 november 2006
- Real-Time PCR - description and applications -1st march 2007
- The use of PCR method for diagnostic and epidemiology of the infectious diseases"– 1-5 October 2007
- Tracing viral infections by using molecular methods (PCR and sequencing) – 24-28 november 2008
- Workshop : „PCR methods", collaboration with Top Diagnostics (20.01.2010);
- Workshop „The role of molecular biology in diagnosis of infectious diseases" in collaboration with Novaintermed (20-21 April 2010)

Publications in ISI journals

- Sultana C., **Oprisan G**., Szmal C, Vagu C., Temereanca A., **Dinu S**.,Teleman MD., Ruta S. Molecular Epidemiology of Hepatitis C Virus Strains from Romania. **Journal of Gastrointestinal and Liver Diseases,** 2011 Sep;20(3):261-6. **Impact Factor: 1.434** http://www.jgld.ro/2011/3/8.html
- Baicus A, Persu A, **Dinu S,** Joffret ML, Delpeyroux F, **Oprisan G**. The frequency and biodiversity of poliovirus and non-polio enterovirus strains isolated from healthy children living in a limited area in Romania. *Arch Virol.* 2011 Apr;156(4):701-6. Epub 2011 Jan 8. (PMID: 21221676)
- Banica Leontina, Besliu Alina, Pistol Gina, Stavaru Crina, Ionescu Ruxandra, Forsea Ana-Maria, Tanaseanu Cristina, Dumitrache Sergiu, Otelea Dan, Tamsulea Isabela, Tanaseanu Stefanita, Chitonu Cristina, Paraschiv Simona, **Balteanu Monica**, Stefanescu Maria and Matache Cristiana. Quantification and molecular characterization of regulatory T cells in connective tissue diseases. *(2009), Autoimmunity,42:1,41-49;*
- M Combiescu, S Guillot, A Persu, A Băicuş, D Pitigoi, J Balanant, **Gabriela Oprisan**, R Crainic, F Delpeyroux, AA Combiescu. 2007. Circulation of a type 1 recombinant vaccine-derived poliovirus strain in a limited area in Romania. Archives of Virology, 2007, 152(4):727-38. Impact Factor – 2.02. Cited by 4.

Publications in Journals indexed in international data banks

1. G. Oprisan, C. Szmal, **S. Dinu**, A.-M. Oprisoreanu, V. Thiers, M. Panait, D. Otelea, P. Mavormara *et al*. Comparative methods for genotyping hepatitis C virus isolates from Romania. **2009**. *Romanian Archives of Microbiology and Immunology*, 68(3), 151-157 (MedLine, PubMed);

2. A. Baicus, A. Persu, M. Popescu, A. Penciu, S. Stavri, A. Soare, N. Grecu, **C. Szmal, G. Oprisan**. Correlation between vaccine coverage against polio and circulation and genetic evolution of the poliovirus strains isolated in Romania in the framework of the global polio eradication strategy. **2009.** *Romanian Archives of Microbiology and Immunology*, 68(3), 145-150 (MedLine, PubMed).

3. Damian M, Tatu-Chiţoiu D, Usein CR, **Oprişan G**, Palade AM, **Dinu S**, Szmal C, Ciontea SA, Ceciu S, Condei M, Persu A, Baicuş A, Pop M, Neagoe I, Steriu D, Codreanu R, Graur M, Cretu MC, Cilievici S, Nica M, Ecovoiu A, Gavrila L. Laboratory diagnosis of infectious diarrhoea syndrome; a three years study in two hospitals of infectious diseases. *Roum Arch Microbiol Immunol*. **2009** Apr-Jun;68(2):89-94. (PMID: 20361527).

4. C. Ceianu, R. Panculescu-Gatej, A. Cotar, N. Andreescu, M. Waldstrom, **G. Oprisan,** *et.* Trace the circulation of some emergent viruses from family Bunyaviridae in Romania. *Bacteriologia Virusologia Parazitologia Epidemiologia* **2009,** 54, 25-26, (MedLine, PubMed);

5. Oprişan G, Dima S, Tănase AM, Dumitraşcu T, **Dinu S**, Szmal C, Mihăilă M, Pineau P şi Popescu I. Mutations in HCV genome and their implications in resistance and carcinogenesis. *Bacteriologia Virusologia Parazitologia Epidemiologia* **2009,** 54, 30-31 (MedLine, PubMed);

6. Panait M, **Oprişan G**, Codiţă I. Progresses in the molecular diagnosis of hepatitis B viral infection. *Bacteriol Virusol Parazitol Epidemiol.* **2008** Jan-Mar;53(1):5-12. (PMID: 19241991);

Abstracts published in ISI journals

1. A. Marchio, A.-M. Tanase, T. Dumitrascu, M. Mihaila, S. Dima, A. Dejean, I. Popescu, **G. Oprisan**, P. Pineau. HIGH CHROMOSOME INSTABILITY AND OCCASIONAL R249S TP53 MUTATION CHARACTERIZE HEPATOCELLULAR CARCINOMA IN ROMANIA. Journal of Hepatology, Volume 52, Supplement 1, April 2010, Pages S229-S230. **ISSN**: 0168-8278

2. A.M. Tanase, P. Pineau, S. Dima, T. Dumitrascu, **G. Oprisan**, A. Marchio, S. Dinu, A. Dejean, I. Popescu. DOES FAL (FRACTIONAL ALLELIC LOSS) ANALYSIS PREDICT OUTCOME IN PRIMARY LIVER CANCER? Journal of Hepatology, Volume 52, Supplement 1, April 2010, Page S23. **ISSN**: 0168-8278

3. Oprisoreanu AM, Szmal C, Thiers V, **Oprisan G**: Comparative analysis of three different regions from HCV for genotyping Romanian strains. 17th ECCMID, Munich, Grermany, 31 March-3 April 2007. International Journal of Antimicrobial Agents, vol 29, Suppl 2, S399-S400. **ISSN:** 0924-8579.

4. MLC Panait, Oprisoreanu AM, Szmal C, Negrea R, Codita I, Molnar G, **Oprisan GR** „Phylogenetic Analysis of a Hepatitis C Viral Nosocomial Outbreak". 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia June 19 - 22, 2008. International Journal of Infectious Diseases, Volume 12, Supplement 1, December 2008, Page e424. ISSN: 1201-9712

5. S. Ruta, C. Sultana, L. Manolescu , G. Tardei, A. Motoc, D. Brehar- Cioflec, C. Szmal, S. Dinu, A.M. Oprisoreanu, **G. Oprisan**. „The Changing Profile of Circulating HCV Genotypes in Romania". 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia June 19 - 22, 2008. Page e94. ISSN: 1201-9712

Congress and conferences

1. Pascal Pineau, Agnès Marchio, Abdelah Akil, Anne Dejean, **Gabriela Oprisan**, Simona Dima, Traian Dumitrascu, Anna-Maria Tanase, Irinel Popescu. En Roumanie, le cancer primitif du foie est caractérisé par une forte instabilité chromosomique et la présence de la mutation R249S du gène de la p53. Les Journee Francophones d'Hepato-gastroenterologie et d'Oncologie Digestive JFHOD 2010, Paris, 25-28 martie 2010.

2. **S. Dinu**, G. Tardei, A. Motoc, M. Straut, **G. Oprisan**, Study of the antiviral therapy induced mutations in the polymerase gene of hepatitis B virus in Romanian patients, **4th Congress of European Microbiologists FEMS , 26-30 iunie 2011, Geneva. Poster**

3. Sorin Dinu, Camelia Szmal, Maria Nica, Carmen Michaela Cretu, Ana Persu, Maria Damian, **Gabriela Oprisan.** Molecular characterization of new identified viruses (Noroviruses) in Romania. 3rd Congress of European Microbiologists FEMS 2009, Gothenburg, Suedia, 28 iunie-2 iulie 2009

4. Oprisan G, Dima S, Tanase AM, Dumitrascu T, Dinu S, Szmal C , Mihaila M, Pineau P and Popescu I. Comparative analysis of the core and NS4B sequences of Hepatitis C Virus from liver tumor and sera. 3rd Congress of European Microbiologists FEMS 2009, Gothenburg, Suedia, 28 iunie-2 iulie 2009.

5 G. Oprisan, C. Szmal, **S. Dinu**, A. Oprisoreanu, V. Thiers. Molecular epidemiology of hepatitis C virus in Romania. The 18th ECCMID, 19-22 April 2008, Barcelona, Spain;

6. **Gabriela Oprisan, Sorin Dinu**, Camelia Szmal, Codruta-Romanita Usein. Molecular tools in tracing HCV infections. Second Congress of Virology (Days of Virology in Bulgaria), 28-31 May 2008, Sofia, Bulgaria;

7. G. Oprisan, V. Thiers, C. Szmal, **S. Dinu**, AM. Oprisoreanu, D. Otelea, P. Maillard, A. Budkowska and P. Mavromara. Evaluation of molecular tools, developed in the frame of the PTR 126, for genotyping and molecular epidemiology of HCV strains in Romania. Conférence scientifique internationale "La recherche sur les maladies infectieuses: un défi planétaire", 26-27 June 2008, Institut Pasteur, Paris ;

8. 12. Ana Persu, Camelia Szmal, Anda Baicus, Luminita Popa, Mariana Combiescu, Andrei Aubert-Combiescu, Sorin Dinu, Radu Crainic, Valerie Caro, Sophie Guillot, Hinda Triki and **Gabriela Oprisan**. „Application of three PCR systems, developed within the framework of the project Entérovirus (RIIPIA), for the identification and the molecular analysis of the untypable enteroviruses and of epidemic echoviruses isolated in Romania". Conférence scientifique internationale "La recherche sur les maladies infectieuses : un défi planétaire", Institutul Pasteur, Paris, 26-27 iunie 2008.

Books, handbooks, chapiters

- **Gabriela Oprişan, Camelia Szmal, Sorin Dinu**, Ana-Maria Oprişoreanu, Mircea Liviu Cătălin Panait (INCDMI Cantacuzino) and the consortium of CEEX 158/2006 project. Investigations of the molecular mechanisms of HCV genome involved in the development of some systems for diagnostic and therapy". Ed. Univ. „Carol Davila", Bucharest, **2008,** ISBN nr. 978-973-708-350-0.
- Dorel Lucian Radu, Manuela Militaru, Aurelia Ionescu, Crina Stavaru, Emilia Lupulescu, Adrian Onu, Melania Grosu, Bogdan Marinescu, **Gabriela Oprisan**, Roxana Dimitriu, Adriana Costache, Iuliana Onita si colab. Viorel Alexandrescu, Margareta Mazilu, Camelia Szmal et. al.: „Studiu experimental si clinic al infectiei gripale si al imunizarii cu vaccinuri gripale", Universitara „Carol Davila", Bucuresti, 2008, ISBN nr. 978-973-708-332-6.

T6 Nosocomial Infections and Antibiotic Resistance

Research team:

Team leader: Codiţă Irina, Senior Researcher I

icodita@cantacuzino.ro; staphylo@cantacuzino.ro; adirina_2005@yahoo.com;

Research staff:

Lixandru Brânduşa – Elena, Senior Researcher III

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4 Drăgulescu Elena-Carmina – Researcher

staphylo@cantacuzino.ro;elena_dragulescu@yahoo.com;

5 Pana Marina (1/4) – Senior researcher II

strepto@cantacuzino.ro; mpana@cantacuzino.ro;

6. Dragomirescu Cristiana Cerasella

dragomirescu_cerasela@yahoo.com;

6 Straut Monica (1/4) – Senior Researcher I

mstraut@cantacuzino.ro;

Other personnel: -

Niculita Dumitra, med ass

Panoschi Cristina, med ass

Mission, objectives, research interest

The main mission of our laboratory is to answer reference function requests rised by nosocomial infections and antimicrobial resistance surveillance and early warning at national and international level.

General objectives: Research projects developed in the last 5 years were designed to implement and optimize new molecular methods in order to strengthen the reference laboratory capacity to detect and trace nosocomial infections, antimicrobial resistant or virulent microorganims alerts etc. Increasing rapid detection and clonal differentiation capacity resulted from technical and scientific progress achieved by developing a national excellence research program (2005-2008) improved our performance at national level and our involvment in European surveillance and research networks.

The second research pilar was directed towards developing national and European multidisciplinary applicative research projects in collaboration with partners specialised in polimers chemistry, rare and nonferrous metals, physics of radiation and lasers, etc. aiming to setting up and validating antimicrobial materials and/or new equipments for medical waste neutralisation.

Specific objectives:

- To trace geographical distribution of *S. aureus* and *Pseudomonas aeruginosa* strains at national level using phenotipical and molecular methods as: PFGE, spa typing MLST typing, SCCmec typing toxinotyping etc.

- To study virulence factors of *S. aureus* and *Pseudomonas aeruginosa* strains in relation with their involvment in hospital and/or community aquired infections: Panton Valentine leucocidine, staphylococcal enterotoxins, *Pseudomonas aeruginosa* virulence factors etc.

- To detect antimicrobial resistance and AMR markers: methicilline resistance and/or *Pseudomonas aeruginosa* beta-lactamases and typing of antimicrobial resistance genes etc.

Research projects:

I. International projects:

1. "Commensalisme et infections urinaires a Escherichia coli multiresistants de part et d'autre de la frontiere de l'Union Europeene" (Commensalism and multi-drug resistant *E. coli* Urinary Tract Infections on both sides of the European Union Boarders): ECO – NET EGIDE Project Nr. 18876QJ financed by the French Foreign Affairs Ministery - collaboration with Hopital Bichat Claude Bernard, Paris
Project director: Prof. Antoine Andremont, Hopital Bichat - Claude Bernard, Paris

Project responsable for INCDMI "Cantacuzino": Codita Irina, Senior Researcher I

Project interval: 2008-2009

Project budget: 2008 – 13,000 Euro; 2009 – 10,000 Euro

INCDMI "Cantacuzino" budget was directly expressed as:

- materials and reagents for sampling and bacterial strains isolation

- one visit of a microbiologist from Bichat Claude Bernard Hospital in our laboratory

- one stage for a young Romanian researcher from our laboratory (Brindusa Elena Lixandru) at the Bichat Claude Bernard Hospital (2009)

Project objective: To quantify the prevalence of ESBL positive *E. coli* in the urinary tract infection isolates (UTI) and to evaluate the importance of the enteric reservoir in patients affected by community UTI in Romania and Republic of Moldova.

Results: ESBL *E.coli* faecal carriage was not a good predictor of ESBL *E.coli* UTI, yet the negative predictive value was high. Surprisingly, in patients with ESBL *E.coli* in both stool and urine the faecal

strain was often not the infecting one. Together with the finding that ESBL *E. coli* relative concentration in faeces was higher in patients with discordant clones than in those with concordant ones it suggests intestinal densities does not play a major role in ESBL *E. coli* UTI pathogenesis. Other factors such as virulence characteristics of the various strains are currently under investigation.

2. **Doctoral fellowships for ecoeconomic and bioeconomic complex training in antropic ecosystems food and fodder biosafety and biosecurity** ("**Burse doctorale de pregatire ecoeconomica si bioeconomica complexa pentru siguranta si securitatea alimentelor si furajelor din ecosisteme antropice") - Proiect POSDRU 107/1.5/S/77082**

Project director: Dr. Ipate Iudith**,** Economic Institute of Romanian Academy

Project responsable for INCDMI "Cantacuzino": Codita Irina, CS I

Project interval: 1.12.2010 – 30.11.2013

Project budget for INCDMI "Cantacuzino"

Total: 1081212.50

CE contribution: 902880,63

INCDMI "Cantacuzino" cofinancing: 159.331, 88

CE contribution: 2011 – 288912,8016 (32%); 2012 – 378424,375 (35%); 2013 – 902.880,63 (33%)

Objectives: The main objective is to improve the professional formation of future researchers using the frame of the doctoral sudies by implementing competitive doctoral curriculum based on interdisciplinary approach and increased transnational mobility, including interventions for facilitating co-tutorship for doctoral studies, with consistent financial support.

The main specific objectives are:

- Increasing the training system capacity in Romania in the field of eco-economic and bioeconomic complex problems for food and fodders security and safety in Romania
- Setting-up a Consortium to support collaboration between universities, research bodies and companies
- Increasing motivation and involvment of young researchers in top research activities, increasing innovation and creativity in the particular field of microbiologic biodiversity and biotechnologies connected with emerging potential zoonoses prevention and control
- Collaboration with regional bodies for emergency situations interventions (BRISU)
- Dissemination of doctoral programs research results (manuals, guidelines, treatise etc.) by using modern tools, including e-learning in the following domains: integration of EU legislation in the food biosafety domain, food safety and security: theory and laboratory methods, food microbiological safety: theory and laboratory methods, good practice guidelines etc.
- Secondary objectives: to include in the same target group young reaserchers from all the eight major domains, who will ensure food resources of the mankind in the XXI century, by respecting bio-economy and eco-economy, traceability, quality and food safety principles.

Results 2011:

We participated in:

- setting up he curriculum or the doctoral school in the food microbiology domain

- setting up the food microbiology theoretical manual and practical work book summaries

- organizing a scientific syposium on food microbiology

National research projects

1.Complex Excellence Project: "Integrated pathogenomic network (platform) for research result translation in the biomedical domain (infectious diseases microbiology)" – CEEX 28/2005

Project director: Irina Codita, Senior Researcher I

Project interval: 2005 – 2008

Project total budget: 1 500 000

INCDMI "Cantacuzino" budget: 733 850

Year	Budgets by years
2005	11350
2006	308000
2007	324000
2008	90500
Total	733850

General objective: To integrate and harmonize national capacities and human expertize in the pathogenomics area

Specific objectives:

- To thoroughly study genetic characterization methods accessible in Romania for selected pathogens and to elaborate the methodology to demonstrate the pathogenomics model functionality (*S. aureus, Enterococcus spp., VHB, Candida albicans, Mycobacterium tuberculosis)*

- To ensure the representativity of strains included in the study

- To validate technical solutions selected by theoretical modeling, practical expertize of project participants and by information exchange with EU pathogenomic platform contact points

- Platform design and initiation of service networks

- To demonstrate the integrated platform functionality

Results:

- Several molecular methods were optimized in our laboratory or institute, as SCC*mec* typing, PFGE typing, spa typing, MLST typing for S. aureus; MLST and erm typing for *Enterococcus* spp., MLST for Candida albicans, spoligotying for *Mycobacterium tuberculosis* etc.

- Preliminary results on geographycal dsitribution of mentioned bacterial and/or viral microorganisms were communicated and/or published

- A pathogenomic network (platform) was set up and it's functionality was demonstrated

2. Cooperation project "New antimicrobial materials to controle nosocomial infections" PP 42 -129/2008 ANTIBIOFILMS - financed by the National Education and Research Ministry of Romania

Project director: National Research-Development Institute for Lasers, Plasma and Radiations Physics **-** Dr. Ing Cristina Surdu – Bob.

Project responsible for INCDMI "Cantacuzino" - Dr. Irina Codita

Project interval: 1.10.2008 – 30.11.2011.

General objective: This project aims to demonstrate the antimicrobial efficacy of different materials covered with Copper and Silver metalic nanofilms and to demonstrate the benefits of using them in hospitals for diminishing the microbial flora in the hospital environment.
Specific objectives:

- To evaluate the antimicrobial activity of small samples covered with Copper or Silver nanofilms
- To evaluate the antimicrobial activity of combined Copper and Silver nanofilms, in different proportions
- To evaluate the effect of using metalic Copper and Silver nanofilms in different proportions of the two metals

Results:
We developed an adapted method to study the antibacterial activity of Copper and/or Silver nanofilm covered surfaces on ***Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Bacillus anthracis*** (Sterne strain) and ***Candida albicans*** based on the Japan Industrial Standard JIS Z 2801: 2000.

Copper-Silver combined nanofilms showed better antibacterial and antifungic activity than simple Copper and Silver nanofilms.

The best antimicrobial activity was obtained with combined Copper/Silver nanfilms, with Copper predominant proportion.

We obtained a partial inhibitory effect on 10^{-7} Enterovirus cell cultures, at 30 ' and a total inhibitory effect at 24 h.

Experiments accomplished at the Cardio-Vascular Emergency Clinical Institute "CC Iliescu", Intensive Care Unit on the effects of placing a textil fabric impregnated with a Copper-Silver nanofilm in the condensation water letting out groove from the air conditioners confirmed the positive antimicrobial effect of the metal nanofilm, reflected in the reduction of the microorganisms number in the hospital room air.

INCDMI "Cantacuzino" Project Budget:

Phase	Phase interval	Phase budget
I	1.10.2008 – 28.02.2009	50000

II	1.03.2009 – 15.12.2009	8500
III	16.12.2009 – 10.12.2010	82299
IV	11.12.2010 – 30.11.2011	101.200
Total		241299

2. **"Cooperation" Project "Developing and bringing up-to date food foils based on polimers and natural antimicrobial agents to increase food safety"– PP 52-134 4/2008 – ACTI-BIO-PACK**

Project director: Institutul National de Cercetare-Dezvoltare pentru Chimie si Petrochimie - Dr. Eng. Liliana Anton

Project responsible for INCDMI "Cantacuzino" - Irina Codita, Senior Researcher I **Project interval**: 2008-2011

General objective:

- - To develop and implement new original, innovative, competitive products for food packaging (biopolimeric antimicrobial foils), based on biologically active molecules from natural regenerable resources incorporated in a polimeric matrix, in order to promote long term development, improvement of food safety and security
- - To develop antimicrobial biodegradable food packaging foils, with environmental reduced impact

Specific objectives:

3. To scientifically prove the strategy for foils developing and testing of bioactive food packaging
4. Theoretical model of antimicrobial foil for food packaging and preliminary microbiological testing
5. Experimental model and setting up of foils preparation and use techologies
6. Chemical analysis of essential oils
7. Physical and mechanical characterization of foils; chemical and physical characterization of foils after the contact with the food sample
8. Testing of the antimicrobial activity of essential oils extracted from aromatic and medicinal plants and of the active foils

Testing of antimicrobial foils after the food contact
Dissemination of results
Patent request from the Romanian Patents Agency (OSIM)

- Results:
- - The essential oils extracted from thyme (Thymus vulgaris L.), basil (Ocimum basilicum L.), coriander (Coriandrum sativum L.), rosemary (Rosmarinus officinalis L.), sage (Salvia officinalis L.), fennel (Foeniculum vulgare L.), spearmint (Mentha spicata L.) and carraway (Carum carvi L.) were investigated for their antimicrobial activity against eleven different bacterial and three fungal strains belonging to species reported to be involved in food poisoning and/or food decay: S. aureus ATCC 25923, S. aureus ATCC 6538, S. aureus ATCC 25913, E. coli ATCC 25922, E. coli ATCC 35218, Salmonella enterica serovar Enteritidis Cantacuzino Institute Culture Collection (CICC) 10878, Listeria monocytogenes ATCC 19112, Bacillus cereus CIP 5127, Bacillus cereus ATCC 11778, Candida albicans ATCC 10231,

Aspergillus niger ATCC 16404, Penicillium spp. CICC 251 and two E. coli and Salmonella enterica serovar Enteritidis clinical isolates. The majority of the tested essential oils exibited considerable inhibitory capacity against all the organisms tested, as supported by growth inhibition zone diameters, MICs and MBC's. Thyme, coriander and basil oils proved the best antibacterial activity, while thyme and spearmint oils better inhibited the fungal species.

- - Patent request deposit

- - Communications and publications

- INCDMI „Cantacuzino" project budget:

Phase	Phase interval	Phase budget
I	1.10.2008 – 16.02.2009	7000
II	-	0
III	15.12.2009 – 15.12.2010	15396
IV	16.12.2010 – 15.11.2011	44098
Total		66494

3. Core project: PN 09 22 0105, **„Rapid methods to detect toxic analits in clinical, food and/or environmental samples"**, as part of Core Program PN 09 22 01, ***„Infectious diseases management: new detection, diagnostic, monitoring methodologies and modern biotechnologies development"*** financed by the National Education and Research Ministery
Program Director – Monica Straut, Senior Researcher I
Project responsible: Irina Codita, Senior Researcher I
Project interval: 2008 – 2011
General objective: to develop rapid, specific and highly sensitive method for detecting microbial toxins in clinical samples, foods and environmental samples

Specific objectives:

- Optimizing PCR for detecting staphylococcal enterotoxin genes in microbial cultures
- Optimizing thermonuclease test and PCR for *nuc* genes to detect S. aureus growth in clinical, food and environment samples
- Using ELFA (Vidas staph enterotoxin II Biomerieux) staphylococcal enterotoxins in microbial cultures, clinical samples, foods and environmental samples
- Otimizing real time PCR to detect staphylococcal enterotoxins genes
- Comparative study of rapid methods, specific and highly sensitive methods used to detect staphylococcal enterotoxins

Project budget:

Phase	Phase interval	Phase budget
I	2.03.2009 - 10.09.2009	50000
II	11.09.2009 – 10.12.2009	50000
III	11.12.2009 – 15.06.2010	40000
IV	16.06.2010 – 10.12.2010	60000
V	11.12.2010 – 15.06.2011	80000
VI	16.06.2011 – 29.11.2011	20000
Total		300000

New project proposals (2011):

1. Microwaves ecofriendly alternative for a safe treatment of medical waste" - Project LIFE10 ENV/RO/000731 – 2 partners from Romania and 1 partner from Bulgaria

Project Director - Dr. Eng. Liliana Sandica Gherghe, National Research-Development Institute for Nonferrous and Rare Metals

Project responsible for INCDMI "Cantacuzino": Codita Irina, Senior Researcher I

Project interval: 1.09.2011 – 30.11.2013 (26 luni)

INCDMI "Cantacuzino" project budget for 26 months:

- CE contrinution: 52724 €
- INCDMI "Cantacuzino" cofinancing: 55377 €

Main objective: Setting up, testing and documenting the certification of an innovative environment friendly device for safely microwaves treating of medical waste

Specific obiectives:

- Reviewing and assessing of law regulations regarding medical waste monitoring at local and national level in Romania and Bulgaria
- Designing, setting up, and testing the pilot sterilising device
- Testing the prototype in real life conditions at the "Cantacuzino"Institute
- Elaborating the documentation for the patent request

2. "Ideas" national project: PN-II-ID-PCE-2011-3-0953 Evaluation of an original alternative to treat osteomyelitis using an animal experimental model

Project responsible: National Institute for Lasers, Plasma and Radiation Physics (INFLPR) - Physician Carmen Cristina Surdu-Bob

Partners fro INCDMI "Cantacuzino": Codita Irina and Coman Cristin

General objective: To assess the antimicrobial effect of metal ions on experimental osteomylitis

Project budget (at INFLPR): 1500000 RON

INCDMI "Cantacuzino" will be payed for services (approx. 600000 RON)

Brevets (national and international):

National brevet request 2011: Composition and procedure for fabrication of food packaging antimicrobial foils

Other relevant activities

Our laboratory is participating in the European Antimicrobial Resistance Surveillance Network (coordinated by ECDC since 2010) and two research networks developed in connexion with the surveillance system: the European Laboratory Network for Sequence Based Typing of Microbial Pathogens and the European Network of Staphylococcal Reference Laboratories.

Irina Codita is nominated as National Focal Point for Antimicrobial Resistance for ECDC and she is also acting as Associated Professor in the Microbiology Chair, "Carol Davila" University of Medicine and Pharmacy, Bucharest.

Publications in ISI journals:

1. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis - Grundmann H, Aanensen DM, van den Wijingaard CC, Spratt BG, Harmsen D, Friedrich AW, European Staphylococcal Reference Laboratory Working Group (… **Monica Straut, Irina Codita….**) - PloS med 2010; 7(1):e1000215; PubMed ID: 20084094

Publications in journal indexed in international data banks - 10

1. New Delhi metallo-beta-lactamase 1-producing *Enterobacteriaceae*: emergennce and response in Europe - Struelens MJ, Monnet DL, Magiorakos AP, Santos O'Connor F, GieseckeJ, European MDM-1 Survey Participants **(…..Irina Codita…**), Euro Surveill. 2010 Nov 18; 15 (46), PubMed :21144431

2. A European laboratory network for sequennce-based typing of methicillin-resistant *Staphylococcus aureus* (MRSA) as a comunication platform between human and veterinary medicine- an update on SeqNet.org - Friedrich A, Witte W, de Lencastre H, Hryniewic W, Scheres J, Westh H, SeqNet.org participants **(….Irina Codita, Monica Straut….),** Euro Surveill. 2008 May 8; 13(19, PubMed ID:18761982

3. Trends in antimicrobial resistance in Europe: update of EARSS results - de Kraker M, van de Sande-Bruinsma N, on behalf of all EARSS participants **(.....Irina Codita....)** - Euro Surveill 2007 Mar; 12 (3), PubMedID: 18976555

4. Antimicrobial activity of plant essential oils against bacterial and fungal species involved in food and/or food decay - **Lixandru BE, Dracea NO, Dragomirescu CC, Dragulescu EC, Coldea IL**, Dobre E, Rovinaru C, **Codita I,** Roum. Arch. Microbiol. Immunol. 2010 Oct-Dec;69(4):224-30, PubMed ID:21462837

5. Antimicrobial activity of Copper and Silver nanofilms on nosocomial bacterial species - **Codita I**, Caplan DM, **Dragulescu EC, Lixandru BE, Coldea IL, Dragomirescu CC**, Surdu-Bob C, Badulescu M. - Roum Arch Microbiol Immunol 2010 Oct-Dec; 69(4):204-12, PubMed ID:21462835

6. Evaluation of antibiotioc resistance in the frame of the surveillance system for nosocomial infection. Strong and weak points - Serban R, **Codita I -** English Abstract, Journal Article:Bacteriol Virusol Parazitol Epidemiol 2010 Apr-Jun 55(2): 169-77, PubMed ID:21553482

7. Trends of antimicrobial resistance in microbial strains isolated from invasive infections in Romania. EARSS participation experience - **Codita I**, Delcaru C, Chifiriuc MC, et al. - English abstract, Journal Article: Bacteriol Virusol Parazitol Epidemiol 2010 Apr-jun; 55(2):151-60, PubMed ID:21553480

8. Antimicrobial resistance surveillance: from the bottle plunged into the ocean to a critical evaluation of the methods - **Codita I**, Serban R, Canton A, et al - English Abstract, Journal Article: Bacteriol Virusol Parazitol Epidemiol 2010 Apr-Jun, 55(2):145-50, PubMed : 21553479

9. *Tricophyton rubrum* in a case of tinea unguium: phenotypic mycological diagnostic algorythm and its limits - Constantin M, Braileanu M, **Dracea ON**, Marinescu B, Coman C, **Codita I.** - English Abstract, Journal Article: Bacteriol Virusol Parazitol Epidemiol 2010 Apr-Jun; 55(2):45-9, PubMed: 21038705

10. Characterization of *Enterococcus faecium* strains by genetic polimorphism study of VNTR loci - F. Pastramă, Virgilia Popa, C. Stoica, **Irina Codita, Vasilica Ungureanu, Monica Ghita**, N. Catană, Daniela Botus, C. Belteghi, G. Răpuntean – Medicamentul veterinar / Veterinary drug, (CNCSIS B239), 2007, 17(4): 129-136

Presentation to the Congress and Conferences – 10

1. Molecular analysis of *Staphylococcus aureus* strains isolated in a newborns unit in 2010 – **Codita I., Dragulescu E.C., Lixandru B.E., Coldea I.L.,** Dinu S. - 21th European Congress of Clinical Microbiology and Infectious Diseases – Milan, Italy, 7-10 May, 2011

2. Foodborn bacterial toxinoses - **Codita Irina**, **Coldea Luminita, Dragulescu Carmina, Lixandru Brandusa** - 5th Conference of the Romanian Association of Medical Laboratories with international participation, Under IFCC and EFCC auspices, Mamaia, 16-19 IUNIE 2010

3. Panton- Valentine Leukocidin Positive Methicillin-resistant *Staphylococcus aureus* Involved in Community and Hospital Infection in Romania - **Irina Codita, C. Dragulescu, C. Szmal, B. Lixandru, L. Coldea**, R. Serban- VIth Balkan Congress of Microbiology, 28-31 October, 2009, Ohrid, Macedonia

4. High prevalence of MDR *Shigella* and *Salmonella* in Africa and Asia and high prevalence of Quinolone resistance in Asia – M. C. Fonkua, R. Pouillot, T.A.H., Le, V. Cao, L., M. Sire, R. Bercion, N. Guessennd, D. Monchy, D.T.N. Tuyet, **I. Codita**, R. Benaissa, F. Randrianirina, P. Courvalin, Perrier-Gross-Claude – conferinta "La recherche sur les maladies infectieuses: un defi planetaire" – "Research on infectious diseases: a global challenge" – June, 27-28th, 2008, Pasteur, Paris, France

5. Phylogenetic Analysis of a Hepatitis C Virus Nosocomial Outbreak - M.L.C. Panait, A.M. Oprisoreanu, C. Szmal, R. Negrea, **I. Codita**, G.R. Oprisan Bucharest (Romania) - 13th International Congress on Infectious Diseases, Infections in Immuncompromised Patients – symposium, Kuala Lumpur, Malaysia, June 19-23, 2008

6. Panton Valentine Leucocidin positive, t008 *spa*-type methicillin-resistant *Staphylococcus aureus* in a Roumanian Western county hospital – **Irina Codita, Mihaela Oprea**, Camelia Szmal, **Elena-Carmina Dragulescu**, Miliana Trasca, Dana Brehar-Cioflec, Gratiana Chicin, Roxana Serban and Adriana Pistol- 18th European Congress of Clinical Microbiology and Infectios Diseases, Barcelona, 19-22 Avril, 2008

7. Molecular characterisation of *Staphylococcus aureus* isolates belonging to two most prevalent spa types recovered from Romanian hospitals during 2006–2007 - **Oprea M., Dragulescu E.C., Coldea I.L., Codita I.**, Szmal C., Straut M. - 18th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, 19-22 Avril, 2008.

8. Characterisation of *Staphylococcus aureus* strains isolated in intensive care Units in Romania, in ian. 2006 - apr. 2007 interval - Mihaela Oprea, **Irina Codita, Carmina Dragulescu**, Monica Straut - Vth Balkan Conference of Microbiology Budva, Muntenegru, October 24-27, 2007

9. Antimicrobial resistance in strains isolated in 2005 by Romanian National Sentinel System for Nosocomial Infection Surveillance - **Irina Codita, Mihaela 0prea, Ana-Maria Petrescu,** Dorina Tatu-Chitoiu, Vasilica Ungureanu, Maria Ghita, Daniela Lemeni, Rodica Dorin, Dana Brehar- Cioflec, Angela Romaniuc and microbiologists from Arad, Bucuresti, Craiova, Galati, Iasi, Sibiu, Timisoara and Suceava hospital laboratories - 17th ECCMID, Muenich, Germany, 31 March-3 April, 2007.

10. Mariana Carmen Balotescu, Ionica, E., **Irina Codita** – Relevance of A 2 phospholipases and mathrix metallproteases in the assessment of the inflammatory process evolution in periodontal disease - 17th ECCMID, Muenich, Germany, 31 March – 3 April, 2007.

T7_Zoonoses and infections due to anaerobic bacteria, parasites and fungi

Team leader: Caplan Dana Magdalena, *PhD*, Senior Researcher (CS I), M.D., brulisa@cantacuzino.ro

Research staff:

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Codita Irina, *PhD*, Senior Researcher (CS I), M.D., Associate Professor, icodita@cantacuzino.ro.

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Neagoe Ionela, PhD student, Scientific Researcher (C.S.), Biologist, Assistant Professor

Tovirnac Victor, Assistant Researcher.

Other personnel:

Vasile-Bugarin Alexandru, MD

Lemeni Daniela, MD

Botoaca Margareta Ana, Senior Nurse

Gavrila Stefana, Senior Nurse

Gubandru Dorina, Senior Nurse

Maria Iosub, Senior Nurse

Cristina Voinea, Senior Nurse

Nitu Paula, Laboratory Technician.

Mission, objectives, research interest

The main objective of our research team is to implement and develop new diagnostic methods for infectious diseases with zoonotic, anaerobic, fungal and parasitic agents. Our activity is also concentrated on building and permanently optimizing diagnostic and treatment algorithms.

Some of our responsibilities are: epidemiological surveys of native or imported parasitic diseases and technical assistance for subordinate laboratories, Quality assurance, continuous training of specialists, lecture and practical works for resident MDs, and cowork in Clinical Laboratory Schedule Panel for resident MDs, and scientific research through national or international programmes according to the needs of current activity. Our research studies generally regard the diagnostic and epidemiology of these infectious diseases, the ddevelopment and practical implementation of the molecular biology methods for laboratory diagnosis and typing.

Our General objectives are:

1. Applying an efficient diagnostic algorithm to increase the chance of correct diagnosis of infections of public health interest;

2 limproving laboratory methods by implementing modern diagnostic methods (PCR) for diagnosis, epidemiology and future treatment strategies;

3. Optimization of specific work procedures in accordance with international standards and their dissemination through training activities in higher education;

4. Collaboration with specialists from hospitals and research institutions involved in infection control and surveillance;

5. Improving the capacity of participating in research projects within international networks for surveillance of infectious diseases of global concern.

Specific objectives:

- Conventional phenotypical and molecular investigations for the purpose of screening or confirmation of various infections of public health interest, like CDAD or parasitic infections.
- Epidemiological studies of infections caused by *Clostridium botulinum* or *Clostridium difficile* as a nosocomial agent;
- Epidemiological studies of infections with *Cryptosporidium sp*., *Giardia lamblia* and *Toxoplasma gondii* in physiological and pathological immunosuppressed subjects.
- Typing methods applied for identification of *Giardia lamblia* and *Cryptosporidium* subgenotypes and establish a correlation between certain subgenotypes and severity of an infection induced by these parasites.
- Improve prenatal diagnosis by increasing the capacity of early diagnosis of maternal infections with *Toxoplasma gondii* or *L. monocytogenes, Brucella abortus*, by serology or by corroborating immunological methods with modern techniques of molecular diagnostic.
- Surveillance of antibiotic sensibility spectrum evolution of zoonotic or anaerobic bacterial strains.

- Contributions to the elaboration of new therapeutic products for the treatment of infectious diseases with bacterial aetiology, like botulism or tetanus.

Research activity

Most of our research projects were focused on increasing the capacity of our laboratories to solve public health urgent or general issues. Our activity aimed to improve the quality of our diagnostic algorithms, to expand and consolidate the laboratory infrastructure and to adopt new strategies for a more rapid, sensitive and specific detection of pathogens.

The main research themes of our team during 2007-2011 were the following:

- *Detection of the pathogenicity properties of zoonotic bacteria involved in human infectious diseases*

Our researchers studied phenotypical characters like macroscopic or microscopic morphology, antibiotic susceptibility spectrum and pathogenicity properties of zoonotic agents like *Pasteurella spp.* or *Leptospira spp.* isolated from pets and their owners. These studies had as a main objective the improvement of prophylaxis and fighting against bacterial zoonosis (PN II 2007 BIOTEHNOLOGY – CNMP 61-034/2007-2010, scientific responsable Dana Caplan).

- *Control of the vertical transmission of pregnancy associated infections*

The research aimed the improvement of the ante-natal diagnosis of infections with *Toxoplasma gondii* or *Listeria monocytogenes* in the pregnant women in real time by harmonizing the serology and molecular biology methods (based on the PCR technology) (Programme: VIASAN – CEEX 164 / 2006-2008, coordinated by Dan Steriu).

- *Diagnostic and epidemiology of parasitic infections.*

An important objective was the molecular identification of *Cryptosporidium sp.* and *Giardia lamblia* in stool samples. The researchers did optimizations of their PCR protocols and evaluated their improved methods by measuring the detection sensitivity resulted in comparison with that of conventional methods like microscopy examinations and antigen detection in feces (DIADI - CEEX 48 / 2005-2008, scientific responsable Dan Steriu).

- *Improvement of the laboratory diagnostic for infections with anaerobic bacteria and development of therapeutic products against infectious diseases with highly pathogenic bacteria like C. botulinum or C. tetani.*

The main activities were focused on improving the quality of diagnostic by developing the infrastructure for molecular techniques of detection or typing for anaerobic strains, by adopting new testing methods and optimizing the existing protocols (NUCLEUS / PN 09-220104/2009-2011, ccoordinated by Monica Straut, scientific responsable: C.S. Ioana Macovei). Our team also participated in research activities which had as a main goal the development of a new generation of specific and more efficient antiserums specific to viral and bacterial highly pathogen agents like *C. botulinum, C. tetani* or *B. Anthracis* (TERASER, PN II, 61-019, 2007-2010, coordinated by Nadia Bucurenci).

- *The improvement of dental prosthetic conditions and the amelioration of the quality of life for the geriatric edentate patients by preventing the deterioration of the prosthetic area.*

The main objective of the studies was the improvement of the quality of life for geriatric edentate patients. Our role was to evaluate the antibacterial activity of lactoferrin and lactofericin on the clinically significant anaerobic strains we isolated from a number of partially or completely edentate patients (IDEI 254/2007 project coordinated by Maria Damian).

6. *Evaluation of biorisk for laboratory personnel.*

The objective was the biorisk assesment at the Cantacuzino Institute. 292 employees were evaluated concerning their state of health and risk coeficients were calculated (VIASAN 173/2006-2008, coordinated by Nicoleta Andreescu).

ISI publications

2010 - ***Clostridium difficile*** **infection** in **Europe**: a **hospital-based survey**, **Bauer MP**, **Notermans DW**, **van Benthem BH**, **Brazier JS**, **Wilcox MH**, **Rupnik M**, **Monnet DL**, **van Dissel JT**, **Kuijper EJ**; **ECDIS Study Group**, **Lancet** 2011 Jan 1;377(9759):63-73.

2009 - S. Ivana, A. Bogdan, I. Ipate, L. Tudor, S. Baraitareanu, A. Tanase, A.N. Popescu, **D.M. Caplan**, M. Danes, *Food safety and botulism toxin*, Rom. Biotehnol. Lett., 14(3), 4390-4394.

2009 - S. Ivana, A. Bogdan, I. Ipate, S. Baraitareanu, A. Tanase, A.N. Popescu, **D.M. Caplan**, M. Danes, *Food microbial contamination – the main danger in the catering type food industry in Romania,* Rom. Biotehnol. Lett., 14(2), 4260-4266.

Publications in journal indexed in international data banks

2011 - Ivana Simona, **Nicoleta Andreescu**, Lucian Ionita, Viorica Chiurciu, Carmen Ionita, Nicodim Fit, Alexandru N. Popescu. Clinical and Laboratory Studies on the Incidence of Leptospirosis in Romania. Bulletin of University of Agricultural Sciences and Veterinary Medicine. Veterinary Medicine Cluj-Napoca, Vol. 68, ISSUE 2.

2010 - S. Ivana, M.Ed. Caplan, Al. T. Bogdan, **D.M.Caplan**, I. Ipate, S. Baraitareanu, C. Ionita, L. Ionita, A. N. Popescu – Virulence genotype of *Pasteurella multocida* strains isolated from atrophic rhinitis in swine – Scientific Works C Series Veterinary Medicine, vol LVI (3-4), pp180-185.

2010 - M.Ed. Caplan, **D.M.Caplan**, S. Ivana – Transmission of *Listeria* spp. by food products from animal origin - Scientific Works C Series Veterinary Medicine, vol LVI (3-4), pp29-33.

2010 - I.Codita, **D.M.Caplan**, E-C Dragulescu, B-E Lixandru, I.L.Coldea, C.C.Dragomirescu, C. Surdu-Bob, M. Badulescu – Antimicrobial Activity of Copper and Silver Nanofilms on Nosocomial Bacterial Species - Rom. Arch. Microbiol. Immunol., 69 (4), pp 204-212.

2010, **Lemeni D.**, Nosocomial *Clostridium difficile* diarrhea – adverse effect of antibiotic therapy, Bacteriol Virusol Parazitol Epidemiol. Apr-Jun; 55(2):141-4.

2010 - **Nicoleta Andreescu**, Simona Ivana, Coman Cristin, Stefana Gavrila, Dorina Gubandru, Radut Mugurel, Simona Panaitescu. Cainele comunitar – rezervor de germeni pentru leptospiroza umana si animala. Revista Romana de Medicina Veterinara, vol. 20, p. 51-54.

2009 – Maria Damian, Dorina Tatu-Chitoiu, Codruta-Romanita Usein, Gabriela Oprisan, Andi Marian Palade, Sorin Dinu, Camelia Szmal, Simona Adriana Ciontea, Stefania Ceciu, Maria Condei**,** Ana Persu, Anda Bãicus, **Mariana Pop**, **Ionela Neagoe**, **Dan Steriu**, Radu Codreanu, Marian Graur, Michaela Carmen Cretu, Suzana Cilievici, Maria Nica, Alexandru Ecovoiu and Lucian Gavrilă. *Laboratory diagnosis of infectious diarrhea syndrome; a three years study in two hospitals of infectious diseases. Rom Arch. Microbiol Immunol, 2:89-94.*

2009 – **Dan Steriu, Mariana Pop, Ionela Neagoe**, *The Management of Vertically Transmitted Pregnancy Associated Infectious Diseases (PAID) by Pregnants and Infants Survey Through New Serology and Molecular Biology Technics and Methods Issuing a Cost-Effective Diagnostic Algorithm – toxoplasmosis, Rev. Rom. de Parazitologie V.XIX pag. 80*

2009 - **D.M.Caplan**, S.Ivana, ME Caplan - *Susceptibility to antibiotics of Bacillus anthracis strains isolated in Romania* - Rom. Arch. Microbiol. Immunol., 68(2), 106-110.

2008 - S.Ivana, A.T.Bogdan, N.Andreescu, **D.M.Caplan**, C.Savu, A.Alecu, I.Ipate, D.Pusta, A.N.Popescu, B.Georgescu - *Study regarding antibioresistance of Salmonella strains isolated from fish flour* – Bulletin UASMV, Veterinary Medicine 65(1), 477-478.

2008 - S.Ivana, A.T.Bogdan, N.Andreescu, **D.M.Caplan**, C.Savu, A.Alecu, I.Ipate, D.Pusta, A.N.Popescu, B.Georgescu - *Diagnosis and treatment of leptospirosis in humans and dogs* – Bulletin UASMV, Veterinary Medicine 65(1), 259-265.

2008 - **Ionela Neagoe**, **Dan Steriu**, Maria Nica, Andreea Toderan, Codruta Usein and Maria Damian. *Application of differential diagnostic methods for Cryptosporidium sp. parasite detection in faeces, Romanian Journal of Parasitology vol XVIII supplement***.**

2008 - Carmen Michaela Cretu, Suzana Elena Clivici, Lidia Elena Lazar, **D. Ianulle Steriu**, Corina Constantin, **Ionela Neagoe**, R Codreanu, Maria Damian. *Immuno-biological and morpho-imaging correlations in parasitic diseases evolving with diarrhoel syndrome, Romanian Journal of Parasitology, vol XVIII supplement, pp. 68-69*

2008 - **Nicoleta Andreescu**, Dana Caplan, Simona Ivana. Leptospiroza la caine si la stapanul acestuia. Lucrari stiintifice Buletin UASVM Iasi, Veterinary Medicine, vol. 51.

2007 Ionela Neagoe, **Dan Steriu, Mariana Pop**, Ioana Cristea, Bugarin Vasile, Nica Maria, Biolan Tatiana, Carmen Cretu, Lidia Lazar, Suzana Cilievici, Radu Codreanu, Andi Palade, Codruta Usein,

Maria Damian. *Laboratory Diagnostic for Cryptosporidium sp and Giardia lamblia infections. Romanian Journal of Parasitology,* vol XVII, supplement.

2007 – Ionela Neagoe, R. Gherman, A. Radulescu, M. Pop, N. Bucurenci, A. Dumitrache, **D. Steriu**. *Determination of a possible congenital Toxoplasmosis by detection specific antigens (NTP-hydrolase) with monoclonal antibodies in pregnant women with seroconversion. Romanian Journal of Parasitology*, vol XVII, 1, pp.43-47.

Books and chapiters in books

D.M.Caplan „**Clinical Bacteriology (Practical Guide for Diagnostic)**" – Ed. TOTAL PUBLISHING, Bucuresti, 2010 (230 pagini) (ISBN 978-606-8003-08-5).

„Listeria" in **„Tratat de Microbiologie Clinica"** (sub redactia Prof.D.Buiuc si Prof.M.Negut), Editia a II-a, Ed.Medicala, Bucuresti, pp 687-692, 2008.

„Listeria" in **„Tratat de Microbiologie Clinica"** (sub redactia Prof.D.Buiuc si Prof.M.Negut), Editia a III-a, Ed.Medicala, Bucuresti, pp 687-692, 2009.

Presentations to the International Conferences/Congresses

S.Ivana, **D.M.Caplan**, N.A.Popescu, A.Bogdan, I.Ipate – Food poisoning-the main danger in the catering type food industry in Romania. Poster at "XII International Congress of Bacteriology and Applied Microbiology", Istanbul 2008 (poz. BP 473, p. 255).

S.Ivana, **D.M.Caplan**, N.A.Popescu, A.Bogdan, I.Ipate – The food safety in food poisoning caused by *Clostridium botulinum.* Poster at "XII International Congress of Bacteriology and Applied Microbiology", Istanbul 2008 (poz. BP 474, page 256).

S.Ivana, **D.M.Caplan**, N.A.Popescu, A.Bogdan, I.Ipate – Achievement of an efficacious treatment scheme in pasteurelosis in man and pet. Poster at "XII International Congress of Bacteriology and Applied Microbiology", Istanbul 2008 (poz. BP 558, p. 292)

S.Ivana, Andreescu N., **Caplan D.M.**, Popescu N.A., Ipate I. - Pet dod and owner leptospirosis. Poster at "IV. National Veterinary Pathology Kongress", Kemer/Antalya, Turkey, 2008.

D.M.Caplan, S.Ivana, M.E.Caplan, G.Popescu, L.Grigore – Therapeutical problems in a case of meningitis with *Listeria monocytogenes*. « 3rd Congress of European Microbiologists FEMS 2009 », Gothenburg, Sweden, 2009 (poz.36)

D.M.Caplan, S.Ivana, M.E.Caplan, A.N.Popescu, I.Ipate – Antibiotic susceptibility of *Bacillus anthracis* strains causing death. « 3rd Congress of European Microbiologists FEMS 2009 », Gothenburg, Sweden, 2009 (poz.35)

S.Ivana, A.Bogdan, I.Ipate, A.Popescu, **D.M.Caplan**, S.Baraitareanu – Pasteurellosis as zoonosis. « 3rd Congress of European Microbiologists FEMS 2009 », Gothenburg, Sweden, 2009 (poz.159).

S.Ivana, A.Bogdan, I.Ipate, A.Popescu, **D.M.Caplan**, S.Baraitareanu – Diagnosis and treatment in leptospirosos to man and pets. « 3rd Congress of European Microbiologists FEMS 2009 », Gothenburg, Sweden, 2009 (poz.160)

S.Ivana, A.Bogdan, I.Ipate, A.Popescu, **D.M.Caplan**, S.Baraitareanu – Classical swine fever (CSF). « 3rd Congress of European Microbiologists FEMS 2009 », Gothenburg, Sweden, 2009 (poz.258)

S.Ivana, A.Bogdan, I.Ipate, A.Popescu, **D.M.Caplan**, S.Baraitareanu - Surveillance of bluetongue in Romania. « 3rd Congress of European Microbiologists FEMS 2009 », Gothenburg, Sweden, 2009 (poz.257)

D.M.Caplan, S. Ivana, M.E. Caplan – Penicillin resistant *Bacillus anthracis* strains isolated from farmers. "The 9th International Symposium – Prospects for 3rd Millenium Agriculture", Cluj-Napoca, Romania, 2010. (pg.361).

The epidemiology of botulism in Romania, D. Dobre, R. Moroti, A. Hristea, V. Arama, **D. Lemeni**, M. Sisiroi, A. Luka (Bucharest, RO), Poster ECCMID Helsinki 16-19 May 2009.

2008 - P103 Lazar L.E, **Steriu D.I**, Cretu C.M, Cilievici S.E, **Neagoe I,** Codreanu R, Damian M, Constatin C.M.), *Neglected intestinal parasites in acute diarrheal disease: a case-control study in Romania,* International Health and Tropical and Medicine 08 -The Annual Meeting of the Royal Society of Tropical Medicine and Hygiene, 17th – 19th September 2008 Brighton – UK.

2008 - P-058: Cretu C.M, Cilievici S.E, Lazar L.E, **Steriu D.I**, Constantin C.M, **Neagoe Ionela**, Codreanu R., Damian M); *Diarrhea of parasitic origin: diagnostic and management.* X-th European Multicolloquium of Parasitology from satellites to microsatellites" 24-28 august 2008, Paris – France.

Cornelia Ceianu, Raluca Panculescu, Ake Lundkvist, P. Heyman, Sanda Hristescu, **Nicoleta Andreescu**, Ani Cotar, Daniela Badescu, H. Zeller. Serologic evidence for hantavirus infectious in Romania. Comunicare. Conferinta Societatii Europene pentru Ecologia Vectorilor (European Society for Vector Ecology), 25-28 martie 2008, Cambridge, UK.

C.Ceianu, D. Badescu, **N. Andreescu**, I. Cristea, A. Cotar, R. Gatej. Forest envirnment as support for the transmission cycles of vector – borne pathogenic agents, with emphasis on recent trends detected in Romania. International Symposium „Forest ecology, mapping and sustainable management". Palace of Romanian Patriarchate, Bucharest, 6-7 Nov.2009.

Radu Tanasa, **Nicoleta Andreescu**, Danut Turcu, Radut Mugurel. Leptospirosis in Romania – laboratory diagnosis and future developments. COST Action B 28 Meetings and Workshop on „Array Technologies", May 3-6, 2010, Istanbul, Turkey.

Ivana Simona, **Nicoleta Andreescu**, Lucian Ionita, Viorica Chiurciu, Carmen Ionita, Nicodim Fit, Alexandru N. Popescu. Clinical and Laboratory Studies on the Incidence of Leptospirosis in Romania. The 10th International Symposium, 29 September – 1 October, 2011, Cluj-Napoca, Romania.

Other relevant activities

Continuous education and training of young researchers: Researchers Olguta Dracea and Ionela Neagoe are PhD students. Researcher Ioana Macovei has participated in 2007 in a training on reference diagnostic methods for anaerobic bacteria at the Pasteur Institute in Paris, France and recently (2011) in a 6 month training on *Clostridium difficile* and CDAD sponsored by ESCMID at the Gregorio Marañon General University Hospital in Madrid, Spain.

The team also organizez training courses credited by College of Physicians.

In collaboration with the Chair of Microbiology, University of Medicine and Pharmacy “Carol Davilla”, our Laboratories organize training programmes for resident doctors (MDs) and other specialists in Microbiology.

Dr. Caplan was a scientific referent for the publication „**Microbiologie Medicala Veterinara**” (autor Simona Ivana), Editura Stiintelor Medicale, 2008.

Dr. Andreescu participated in training programmes for veterinary doctors (Romvac 2009, 2010, 2011, Pasteur Institute Bucharest in 2010, Fac. Of Veterinary Medicine, Bucharest, 2010).

T8_Bacterial enteric infections

Head of team: Anca ISRAIL, MD, PhD, senior researcher (CSI), ancaisrail@yahoo.com

Research staff: Chifiriuc Mariana Carmen, MS, PhD, senior researcher (CS II), Associate professor University of Bucharest, Faculty of Biology, Microbiology Department, carmen_balotescu@yahoo.com

Daniela Cristea, Sci, scientific researcher (CS III), PhD student

Dracea Olguta, Scientific researcher CS III, PhD student (olgutza_dracea@yahoo.co.uk)

Babes Camelia (camelia_babes@yahoo.com), Research assistant, (until 31 December 2009)

Larion (Delcaru) Cristina, CSIII, biolog specialist, PhD student, larioncristina@yahoo.com) (until February 2010)

Iordache Carmen, Sci, scientific researcher (CSIII), 0.5 , PhD student

Cristina Dinu, senior researcher (CS III)

Stefania Ceciu, Sci, scientific researcher, (until July 2009)

Negut Marian Md, PhD. Prof, CS I (retired, until 2010)

Ani Ioana Cotar, CSIII, PhD (aniioana@yahoo.com)

Other personnel:

Tatu-Chitoiu Dorina, MD, dtatu@cantacuzino.ro

Gabriel Ionescu, MD, PhD

Ciontea Simona, biologist

Iordache Silvia, medical assistant

Popa Andrei, medical assistant

Soricila Geta, medical assistant

Dinca Gherghina, laboratory technician- retired since august 2011

Andrei Melania Mihaela, laboratory technician

Mission, objectives, research interest

- The Bacterial Enteric Infections team is grouping Reference activities for *Salmonella, E. coli, Yersinia, Shigella* and *Vibrio* infections. The main tasks of the team consist in:
- Diagnosis and surveillance of enteric pathogens;
- Development and implementation of the new molecular methods used in diagnostic and surveillance;
- Studies on genetic structures coding for virulence in enteric bacteria;
- Role of some enteric taxonomic groups in nosocomial infections;
- Identification and characterization of mechanisms involved in the antibiotic resistance;
- Studies concerning the antimicrobial activity of new synthesized compounds.

Research activity

1. Improve the diagnosis of digestive infections in order to reduce the reports of diarrhoea with un-known aetiology;

Project CEEX 47 (financed by National Authority for Scientific Research)**:** "Management and diagnosis of diarrheic infection syndrome" – director Maria Damian (**Dorina Tatu-Chitoiu, Simona Ciontea**). The enteric pathogens strains (EPEC, EHEC, *Campylobacter*, etc) isolated from the stools of BDA diagnosed patients were exo-enzymatically characterized and serologically typed; their potential of producing ESBL and AmpC was also tested; finally, the strains were stored for genetic studies.

Project CEEX 143 (financed by National Authority for Scientific Research)**:** "The evaluation of ethyological role of emergent microorganisms in human infections. Molecular and phenotype markers used in their monitoring" – director Maria Damian (**Dorina Tatu-Chitoiu, Simona Ciontea, Ceciu Stefania**). *E coli* and *Klebsiella* strains isolated from extra-intestinal pathological samples (sputa, urine, bronchial aspirate, etc) were tested for their potential of producing ESBL and their sensibility to quinolones and fluoroquinolones.

2. Studies on antibiotic resistance and development of new compounds with antimicrobial activity

Project PN 06150104 (funded by National Authority for Scientific Research)**:** "Resistance to Fluoroquinolones of the Clinical Isolates of Enterobacteria : molecular mechanisms and pathogenic potential correlations" – director Codruta-Romanita Usein (**Dorina Tatu-Chitoiu, Simona Ciontea, Ceciu Stefania**) focused on the phenotypic and molecular characterization of E.coli and Klebsiella pneumoniae strains isolated from extra-intestinal pathological samples, resistant to quinolone and fluoroquinolone. The research concerning the synthesis and characterization of new compounds which could be used as antimicrobial agents were developed in collaboration with University of Bucharest and Faculty of Pharmacy Bucharest within the p**roject PNII 41-043/2007 "**Consortium in the purpose of the study of synthesis, physical and chemical features and biologic activity of new thioureides with potential antimicrobial activity" (Project Director: Associate Prof. Dr. Carmen Limban, INCDMI **scientific responsible Anca Israil**) the **project 41-055/2007** "Multidisciplinary research concerning the synthesis, physical and chemical characterization and antimicrobial activity evaluation of some sulphones with dibezothiepinic structures" (Project Director: Associate Prof. Dr. Camelia Stecoza, INCDMI **scientific responsible Anca Israil**) and **42-095/ 2008** „Studies on synthesis, fizico-chemical characterizationand antimicrobial activity of some new compounds with tricyclic structure" (Project Director: Associate Prof. Dr. Carmen Limban, INCDMI **scientific responsible Anca Israil**)).
The specific objectives of the Project **42-150/2008** „Interdisciplinary research on antibioresistance and the major implications in the pathology of cardiovascular and gastrornteric surgery" (project **director Anca Israil**) were the studies on the bacterial etiology involved in abdominal surgical emergency, bacterial virulence and antibioresistance.

3. Sudies on biofilm and Quorum Sensing mechanisms

Sudies on intra and inter cellular communication of bacterial cells responsible for cellular density monitoring and regulation pathways of different vital functions as a perspective in prevention and treatment of infections are the main objectives of the **project CNCSIS 1312/2008** "Evaluation and monitorization of the soluble mediators implicated in Quorum Sensing mechanisms concerning the control of bacterial multiplication" (**director Anca Israil**). The soluble mediators of Quorum Sensing

produced by different species of *Vibrionaceae* were characterized and their role as auto-inductors was studied.

4. New genetic markers used for microbial identification and characterization

In collaboration with Molecular Microbiology and Epidemiology team our research activity focused on identification of new molecular markers used in diagnosis and epidemiology of food borne infections (**project 42-106**, director Maria DAMIAN), virulence factors characterization and genetic structures coding for resistance to the antibiotics of commensally bacteria producing diarrhea in children under 5 years (**project 42-049,** scientific responsible Maria DAMIAN) and molecular detection of infections due to *Helicobacter pylori* and caracteriation of strains in order to find some correlations between gastric disease and virulence profile of strains involved (**project 42-155,** director Maria DAMIAN).

Human resources training

2 PhD thesis during the research projects:

- Ani-Ioana Cotar, UNIV. BUC., FAC. BIOL. 2010„ Implications of Quorum sensing and response phenomenon in the coordinated expression of the pathogenicity and virulence factors in *S. aureus* and *Ps. aeruginosa* strains" –CNCSIS 1312
- Otilia Banu, INBCV Prof. CC. Iliescu, mai 2010 – „Study of interrealtions between the infectious agenst-eukariotic cells- prosthetic devices in the cardiovascular infections pathology" –PN2 42150.

3 PhD thesis in preparation during the research projects: **CSIII Cristina Larion –Delcaru PN2 42150**, **CS III Olguta Dracea PN2 41043, 41 055 and CSIII Carmen Iordache – CNCSIS 1312**

Member of the PhD Thesis evaluation comissions (**Anca Michaela Israil**):

53. Monica Teleman, UMF Bucuresti, 2011 „Emergent viral infections with nosocomail potential, with special reference to SARS epidemic".
54. Camelia Truica, UMF Bucuresti, 2010, „Prevalenta si incidenta infectiei cu virus hepatitic C in populatia selectata factori de risc in infectia cu virusul hepatitei C".
55. Khadi Alhaddad, UMF Bucuresti, march 2009 „Persistent urinary infections and their implication for the public health".
56. Magda Mitache, DSP Bucuresti, march 2009. „ Research on the specific procedures for the mcirobiological control of the sanitary units in concordance with ISO standards".
57. Caliopsia Florea, Pediatrics Hospital, Galati, mai 2008- Phenotypic and molecular typing of uropathogenic E. coli strains isolated from infants.

- Florian Silviu Alecu, march 2007 – „Implications of *Candida* genus on oral pathologya".
- Jemina Benga, UNIV. BUC., FAC. BIOL., november 2007 – „Viral oncogenes as therapeutic targets for HDV-8 associated malignancies"

- **Supervising of 4 dissertation and graduation thesis (Anca Israil, Carmen Chifiriuc)**

  Larion Cristina – dissertation thesis

  Gratiela Pircalabioru- graduation thesis

Arama Mihaela- graduation thesis

Stancu Roxana- graduation thesis

Sorin Dinu- graduation and dissertation thesis

Participation to workshops

- Anca Israil, Workshop Bioterrorism, Tulcea, September 2009
- Larion Cristina, Workshop EARSS, Bilthoven, Netherland, June 2009
- Curs-„The use of PCR technique in the diagnosis and epidemiology of the infectious diseases" 24 – 28 - September 2007, INDCMI "Cantacuzino", Bucharest: biol. Ciontea Adriana-Simona, biol. Dana Cristea
- Symposion "Information European day on Antibiotics "Jacques Monod" Teaching Centre INCDMI Cantacuzino, Bucharest, 18 nov. 2009 – participants: dr. Dorina Tatu-Chitoiu, biol. Ciontea Adriana Simona, Daniela Cristea, Anca Michaela Israil, Cristina Delcaru
- "SSI international Course of Salmonella serotyping; **4-6 March 2009, Copenhagen**- biol. Ciontea Adriana Simona
- Syumposion Nova Intermed**, 20-21 April 2010, INCDMI" Cantacuzino**" Bucharest -participants : dr. Dorina Tatu-Chitoiu, biol. Adriana Simona Ciontea, biol.Daniela Cristea
- Postuniversitary training course: "Study of the nosocomial and communitary infectious agenst ressitance mechanisms in automatic identification systems and antibiogram" –Collaboration with BioMerieux-France and Mediclim, entitled "Bacterial resistance detection a new challenge for the laboratory to fight HAI !"; **16-18 May 2011, INCDMI"Cantacuzino**", Bucharest.- participants: Dr.Dorina Tatu-Chitoiu, biol. Ciontea Adriana Simona, As.med.Popa Andrei, As. med. Andrei Melania

Exams

1. Adriana –Simona Ciontea : - principal biologist - May 2009

2. Cristea Daniela: specialist biologist - May 2009

3. Cristea Daniela: researcher CSIII -2010

4. Olguta Dracea: researcher CSIII -2010

5. Larion Cristina: researcher CSIII -2010

ISI publications

10. Limban C, Marutescu L, Chifiriuc MC Synthesis, spectroscopic properties and antipathogenic activity of new thiourea derivatives.. Molecules. 2011 Sep 6;16(9):7593-607.
11. Israil AM, Delcaru C, Palade RS, Chifiriuc C, Iordache C, Vasile D, Grigoriu M, Voiculescu D Bacteriological aspects implicated in abdominal surgical emergencies..Chirurgia (Bucur). 2010 Nov-Dec; 105(6):779-87.

12. Chifiriuc MC, Banu O, Bleotu C, Lazar V Interaction of bacteria isolated from clinical biofilms with cardiovascular prosthetic devices and eukaryotic cells. Anaerobe. 2011
13. Cotar AI, Chifiriuc MC, Dinu S, Bucur M, Iordache C, Banu O, Dracea O, Larion C, Lazar V. Screening of Molecular Virulence Markers in Staphylococcus aureus and Pseudomonas aeruginosa Strains Isolated from Clinical Infections. Int J Mol Sci. 2010;11(12):5273-91. Epub 2010 Dec 21.
14. Müller J, Limban C, Stadelmann B, Missir AV, Chirita IC, Chifiriuc MC, Nitulescu GM, Hemphill A.Thioureides of 2-(phenoxymethyl)benzoic acid 4-R substituted: a novel class of anti-parasitic compounds. Parasitol Int. 2009 Jun;58(2):128-35. Epub 2008 Dec 25.
15. Mariana Carmen Chifiriuc, Gratiela Pircalabioru, Beatrice Gîlea, Veronica Lazar, Luminita Dascalu, Gerard Enache, Coralia Bleotu. Immunogenicity of different cellular fractions of *Vibrio parahaemolyticus* strains grown under sub-lethal heat and osmotic stress. African Journal of Microbiology Research Vol. 5(1) pp. xxx-xxx, 4 January, 2011
16. Carmen Limban, Alexandru V. Missir, Ileana C. Chirita, George M. Nitulescu, Miron T. Caproiu, Mariana C. Chifiriuc, Anca M. Israil. Synthesis and antimicrobial properties of new 2-(4- ethylphenoxy)methyl)benzoylthioureas. Chemical papers, 2011 65 (1) 60–69
17. George M. Nitulescu, Constantin Draghici, Mariana C. Chifiriuc, Luminita Marutescu, Coralia Bleotu, Alexandru V. Missir. Synthesis and antimicrobial screening of N-(1-methyl-1Hpyrazole- 4-carbonyl)-thiourea derivatives. Med Chem Res., 2010
18. Mariana Carmen Chifiriuc, Camelia Stecoza, Olguta Dracea, Cristina Larion, Anca Michaela Israil. Antimicrobial activity of some new O-acyloximino-dibenzo[b,e]thiepins and O-acyloximino-dibenzo[b,e]thiepin-5,5-dioxides against planktonic cells, RBL, Vol. 15, No.2, 2010, 5134-5139
19. Carmen Limban, Alexandru-Vasile Missir, Ileana Cornelia Chiriţă, Rodica Guţă, Doina Nănău-Andreescu, George Mihai Niţulescu, Constantin Drăghici, Miron T. Căproiu, Cristina Delcaru and Mariana Carmen Chifiriuc. Synthesis, structural characterization and microbiological assays of some new 2-methoxy-O-acyl-oximino-dibenz[b,e]oxepins. Tome 55, No 6 Juin 2010
20. Diana Camelia Nuţă, Carmen Balotescu Chifiriuc, Alexandru Vasile Missir, Ileana Cornelia Chiriţă, Carmellina Daniela Bădiceanu. *In Itro* Evaluation Of The Antibacterial And Antifungal Activity Of Some New N-(2-Dialkylaminoethyl) Benzanilides FARMACIA, 2010, Vol. 58, 1, 38-45
21. Claudia Mladin, Mariana-Carmen Chifiriuc, Andi-Palade, Codruţa-Romaniţa Usein, Carmen Luminiţa Slavu, Rocsoreanu Alina, Maria Damian Phenotypic and genetic analysis of the antibiotic resistance patterns in uropathogenic *Escherichia coli* strains. RBL, vol. 15. nr. 2/ 2010, p. 5078-5086
22. Claudia Mladin, Codruţa-Romaniţa Usein, Mariana- Carmen Chifiriuc, Andi-Palade, Carmen Luminiţa Slavu, Marian Negut, Maria Damian. Genetic Analysis Of Virulence And Pathogenicity Features Of Uropathogenic *Escherichia Coli* Isolated From Patietns With Neurogenic Bladder" RBL, nr. 6/ 2009

23. Tarko, L., Stecoza, C.E., Ille, C., Chifiriuc, M.C. *QSAR studies on antibacterial activity of some substituted dihydrodibenzothiepins.* Rev. Chimie Volume 60, Issue 5, 2009, Pages 476-479
24. Codruta-Romanita Usein, Dorina Tatu-Chitoiu, Simona Ciontea, Maria Condei, Maria Damian. "Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Japanese Journal of Infectious Diseases 62 (4): 289-293, 2009
25. Stecoza, C.E., Căproiu, M.T., Drăghici, C., Chifiriuc, M.C., Drăcea, N.O. Synthesis, characterization and antimicrobial activity evaluation of some new derivatives of 6,11-dihydrodibenzo[b,e]thiepin 5,5-dioxide. Revista de Chimie. Volume 60, Issue 2, February 2009, Pages 137-141
26. Anca-Michaela Israil, Mariana- Carmen Chifiriuc, Cristina Delcaru, Carmen Iordache, Elena Sasarman. Evaluation And Monitoring Of Quorum Sensing Soluble Mediators Implicated In The Regulation Of Bacterial Growth In *Vibrio* Strains. *Romanian Biotechnological Letters, Vol. 14, No. 2, 2009, Pp. 4211-4224*
27. Anca-Michaela Israil, Cristina Delcaru, Mariana –Carmen Balotescu Chifiriuc. Impact of different parameters upon the expression of certain virulence factors of nonhalophilic and halophilic *Vibrio* strains. *Romanian Biotechnological Letters, Vol. 14, No. 4, 2009, pp. 4545-4559*
28. Cotar A., Chifiriuc C., Dinu S., Bucur M., Iordache C., Banu O., Dracea O., Larion C., Lazar V., 2008, Screening of Molecular Virulence Markers in Staphylococcus aureus and Pseudomonas aeruginosa Strains Isolated from Clinical Infections. *Int. J. Mol. Sci.* 2008, 9, 1-x manuscripts (in press), ISSN 1422-0067, www.mdpi.org/ijms
29. Limban C., Balotescu M.C., Missir A.V., Chiriţă II. C., Bleotu C., 2008, Antimicrobial Activity of Some New Thioureides Derived from 2-(4-Chlorophenoxymethyl)benzoic Acid , *Molecules*, vol. 13, p. 567-580
30. Chifiriuc M.C., Larion C., Iordache C., Lixandru M., Dracea O., Bleotu C., Bucur M., Israil A., 2008, Influence of soluble mediators upon the expression of different physiological and virulence hallmarks of bacteria, *Roum. Biotech. Lett.*, vol. 13, no. 2, p. 3631-3642
31. Limban, C., Missir, A.-V., Chiriţă, I.C., Bădiceanu, C.D., Drăghici, C., Balotescu, M.C., Stamatoiu, O. New thioureides of 2-(4-methyl-phenoxymethyl)-benzoic and 2-(4-methoxy-phenoxymethyl)-benzoic acids with biological activity. *Revue Roumaine de Chimie*. Volume 53, Issue 8, August 2008, Pages 595-602
32. Chifiriuc C., Lixandru M., Iordache C., Bleotu C., Larion C., Dracea O., Lazar, V., Antohe F., Israil A., 2008, Internalization of Staphylococcus aureus and Pseudomonas aeruginosa bacterial cells by non-phagocytic, epithelial human cells, *Rom. Biotehn. Letters*, vol. 13, no. 2, p. 3651-3658.
33. Cotar A., Dinu, S., Chifiriuc, C., Banu, O., Iordache, C., Larion, C., Bucur, M., Dracea, O., Lazar, V., 2008, Screening of molecular markers of quorum sensing in Pseudomonas aeruginosa strains isolated from clinical infections, *Rom. Biotehn. Letters,* vol. 13, no. 3, p. 3765-3770.

34. Cotar A., Dinu S., Chifiriuc C., Banu O., Iordache C., Lixandru M., Dracea O., Bucur M., Lazar V., 2008, Molecular markers of quorum sensing and virulence genes in Staphylococcus aureus strains isolated from biofilm associated infections, *Rom. Biotehn. Letters,* vol. 13, no. 13, p. 3771-3778.
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Abstracts published in ISI journals

9. O. Dracea °, C. Balotescu-Chifiriuc, C. Bleotu, C. Iordache, A. Stanciuc, C. Delcaru-Larion, O. Banu, V. Lazar The Investigation Of The Correlations Between Antibiotics And Host Immune Effectors On Virulence And Antibiotic Resistance Of Some Escherichia Coli Strains, 19th European Congress Of Clinical Microbiology And Infectious Diseases, Helsinky, Finland, 16-19 May, 2009, , R2120 Published In Clin. Microbiol. Infection, 15, S4, P S624, 2009, Print Issn: 1198-743x; Online Issn: 1469-0691; Impact Factor: 3.554

Publications in journals indexed in the international data banks

10. Maria Damian , Codruta –Romanita Usein, Andi Marian Palade, Madalina Baltoiu, Maria Condei, Simona Ciontea, Dorina Tatu-Chitoiu.„Resistance of enteric pathogens to fluoroquinolones and ultimate generation of cephalosporines” ; Bacteriol. Virusol. Parazitol.Epidemiol. 2011, 55(2): 121-129
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12. Mariana Carmen Chifiriuc, Coralia Bleotu, Luminiþa Mãruþescu, Dana Cristea, Veronica Lazãr. THE MODULATION OF HELA CELLS SECRETORY PATTERNS BY INVASIVE *SHIGELLA SPP*. AND ENTEROINVASIVE *E. COLI* BACTERIAL CELLS AND THEIR SOLUBLE COMPONENTS. *Roum. Arch. Microbiol. Immunol*. 2010, 69 (3) 139-145
13. Mariana Carmen Chifiriuc, Coralia Bleotu, Gratiela Pîrcãlãbioru, Anca Michaela Israil, Sorin Dinu, Simona Maria Rutã, Camelia Grancea, Veronica Lazãr. CYTOKINE PROFILES OF HELA AND HUMAN DIPLOID CELLS INDUCED BY DIFFERENT FRACTIONS OF *VIBRIO*

PARAHAEMOLYTICUS CULTURES EXPOSED TO STRESS CONDITIONS. Roum. *Arch. Microbiol. Immunol*. 2010, 69 (3), 154-164

14. Olguta Drãcea, Camelia Babeº, Carmen Limban, Cristina Delcaru, Mariana Carmen Chifiriuc, Anca-Michaela Israil. ANTIMICROBIAL ACTIVITY OF SOME NEW OF 2-(4- ETHYL - PHENOXIMETHYL) BENZOIC ACID THIOUREIDES AGAINST PLANKTONIC CELLS. *Roum. Arch. Microbiol. Immunol*. 2010, 69 (2), 90-95
15. Maria Damian, Dorina Tatu-Chitoiu, Codruta-Romanita Usein, Gabriela Oprisan, Andi-Marian Palade, Sorin Dinu, Camelia Szmal, Simona Adriana Ciontea, Stefania Ceciu, Maria Condei, Ana Persu, Anda Baicus, Mariana Pop, Ionela Neagoe, Dan Steriu, Radu Codreanu, Marian Graur, Michaela Carmen Cretu, Suzana Cilievici, Maria Nica, Alexandru Ecovoiu and Lucian Gavrila.. "Laboratory diagnosis of infectious diarrhoea syndrome; a three years study in two hospitals of infectious diseases".; Rom. Arch. Microbiol. Immunol. 2009 , 68 (2):89-94
16. Codruta-Romanita Usein, Andi-Marian Palade, Dorina Tatu-Chitoiu , Simona Ciontea, Stefania Ceciu, Maria Nica, Maria Damian. Identification of plasmid-mediated quinolone resistance qnr-like genes in Romanian clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*." Rom. Arch. Microbiol. Immunol. 68(1): 55-57, 2009.
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18. Andi-Marian Palade, Codruta-Romanita Usein, Stefania Ceciu, Simona Ciontea, Dorina Tatu-Chitoiu, Maria Damian. "Pulsed-field gel electrophoresis associated to phage typing improves the discrimination of epidemiologically unrelated *Salmonella enterica* serovar Typhimurium isolates. Rom. Arch. Microbiol. Immunol. 68 (2): 42-47, 2009. ted Infections. The Open Epidemiology Journal, 2*:* 69-78, 2009.
19. Codruta-Romanita Usein, Dorina Tatu-Chitoiu, Simona Ciontea, Maria Condei, Maria Damian. "Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Japanese Journal of Infectious Diseases 62 (4): 289-293, 2009.
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2. Cristina Delcaru, RS Palade, Mariana Carmen Chifiriuc, Anca Michaela Israil. Virulence factors in anaerobic bacteria associated with infections in clinical cases of surgical abdominal emergencies. *4th Congress of European Microbiologists*. FEMS 2011, Geneva, Elventia, 26-30 Iunie

3. Alexandra–Maria Nascutiu, Madalina Baltoiu, S.Dinu, Dorina Tatu-Chitoiu, Simona Ciontea, Maria Damian.-Carol davila University of Medicine and Pharmacy ; NIRDMI »Cantacuzino » Bucharest, Romania.
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5. Carmen Limban, Alexandru-Vasile Missir, Ileana Cornelia Chirita, George Mihai Nitulescu, Constantin Draghici, Miron T. Caproiu, Mariana Carmen Chifiriuc, Olguta Nicoleta Dracea. New 2-(4-Ethyl-phenoxymethyl)benzoic Acid Thioureides. Synthesis, Spectral Analyses and Microbiological Assays. *Article first published online*: 5 AUG 2010, ChemInform **Volume 41, Issue 35,** 2010

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16. Balotescu C., Codita I., Radulescu A., Radu S., Floarea I., Larion C., Israil, A.M., 2008, Microbiological, Biological And Clinical Evaluation Of A New Immunostimulatory Strategy Applied In The Treatment Of Periodontal Diseases *3rd FEBS Congress & 11th IUBMB* Conference, Atena, 30 June-4 th July
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23. Gheorghe M.C., Pispiris R.E., Larion C., Iordache C., Dracea O., Lixandru, M., Cotro A., Balotescu M.C., Israil A.M., 2007, Isolation and purification of Quorum-Sensing autoinductors used in regulation of multiplication rate in Vibrionaceae culture media. *31st International Symposion on HPLC and Related Techniques*, June, 2007

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15. M. Neguţ, Maria Damian**, Dorina Tatu-Chiţoiu**, Alexandra-Maria Năşcuţiu, **Simona Ciontea**, Mădălina Băltoiu. Foodborne infections produced by Salmonella. In Maria Damian, Marian Negut. Laboratory diagnostic of food borne infections producedc by Gram negative bacilli" Ed. Ceres, 2011.

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- Brevet OSIM nr. 122638 (2007). "Tioureide N,N'-disubstituite ale acidului 2-(4-fluorofenoxi metil)-benzoic si procedeu de preparare a acestora" (Thioureides N,N'-disubstitution of 2-(4-fluorophenozy-metil)-benzoic acid and procedure of preparation). Limban C., **Balotescu C.**, Chirita I., Missir G., Bleotu C.
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T9_Innate Immunity/ Inflammation/ Immunomodulation

Team leader: Aurora Salageanu, PhD

Research members:

Aurora Salageanu	PhD, Senior Researcher, Head of Laboratory	CS1
Lidia Cremer	PhD, Senior Researcher, Head of Laboratory	CS2
Ana Calugaru	PhD, Senior Researcher	CS2
Iuliana Caras	PhD, Researcher	CS3
Andreea Lupu	PhD, Researcher	CS3
Natalia Apetrei	Researcher	CS
Catalin Tucureanu	Researcher	CS
Iuliana Francisca Anghelache	Researcher	CS
Ramona Cerasela Caragheorgheopol	Researcher	CS
Vlad Constantin Tofan	Research assistant	AS
Camelia Tabarta	technician	T1
Victoria Ivascu	medical assistant	NRDS
Florica Chioseolu	Laboratory technician	NRDS
Stefania Dascalu	medical assistant	NRDS
Lucia Nedu	Laboratory technician	NRDS
Ileana Serbanescu	Laboratory technician	NRDS

Main fields of research:

Innate Immunity

Main research topics:

I. Innate immunity and inflammation in human pathology

The aims of research were: (1) to assess correlations between the pattern of immune mediators and clinicopathological features for patients with colon cancer and larynx squamous cell carcinoma undergoing surgery; (2) to investigate the pathological links between cancer and inflammation.

Multiplex analysis of cytokines (IL-6, IL-8, IL-10, TNF-α, IFN-γ) chemokines (MCP-1, MIP-1α and ENA-78) and growth factors (VEGF and bFGF) in the serum of patients with laryngeal cancer and healthy controls was performed using xMap technology. Patients with SCC presented an altered cytokine profile as compared to healthy controls, both preoperatively (higher levels of IL-8 and IL-10) and postoperatively (higher values for IL-6, IL-8, IL-10 and TNF-α). Heavy smoking was associated with significantly lower levels of ENA-78 and higher levels of IL-8. An inflammatory pattern could be described for colorectal cancer patients before surgery, with elevated and positively correlated circulating levels of IL-6, IL-8, IL-10, TNF-alpha, MCP-1 and ENA-78, which might be explained, at least partially, by the tumor presence. Differences noticed in patients immune mediators profiles suggest that inflammation plays an important role in laryngeal cancer. (Study performed in collaboration with “Sf. Maria” Hospital, “Prof. Dr. Al. Trestioreanu” Oncology Institute, Emergency University Hospital, Bucharest)

We investigated the effect of primary tumor cell supernatants from colorectal cancer pacients on M1/M2 polarization of human peripheral blood monocytes and THP-1 cell line cells differentiated with phorbol myristate acetate (PMA). The cytokine secretion patterns of these macrophages suggest that primary tumor cell supernatants are able to switch the macrophage phenotype or to induce functional polarization of macrophages toward a mixed M1/M2 phenotype.

ISI Publications:

- Melinceanu L, **Lerescu L, Tucureanu C, Caras I, Pitica R,** Sarafoleanu C, **Salageanu A.** "Serum perioperative profile of cytokines in patients with squamous cell carcinoma of the larynx", *J Otolaryngol Head Neck Surg*. 40(2):143-50, 2011
- **Caras I, Tucureanu C, Lerescu L, Pitica R,** Melinceanu L, Neagu S, **Salageanu A**. "Influence of tumor cell culture supernatants on macrophage functional polarization: in vitro models of macrophage-tumor environment interaction". *Tumori*,97:667-74, 2011

Other relevant publications:

- Rugina M, **Caras I**, Jurcut R, Jurcut C, **Serbanescu F, Salageanu A**, Apetrei E, „Systemic Inflammatory Markers in Patients with Aortic Sclerosis", *Roum Arch Microbiol Immunol*. 2007, 66(1-2), 10-16, ISSN 1222-3891, PMID: 18928057 [PubMed - indexed for MEDLINE]

- **Lerescu L, Tucureanu C, Caras I**, Neagu S, Melinceanu L, **Salageanu A**, "Primary cell culture of human adenocarcinomas - practical considerations", *Roum Arch Microbiol Immunol.* 2008, 67 (3-4), 55-66, ISSN 1222-3891, PMID: 19496473 [PubMed - indexed for MEDLINE]

- **Lerescu L, Tucureanu C, Caraş I, Pitica R, Ungureanu V, Sălăgeanu A**, "Involvement of soluble mediators of inflammation in the pathogenic agent interaction-immune system in acute bacterial meningitis", *Bacteriol Virusol Parazitol Epidemiol.* 2008 53(2), 89-97, ISSN 0301-7338, PMID: 19856847 [PubMed - indexed for MEDLINE]

- Melinceanu L, **Lerescu L, Ţucureanu C, Caraş I, Sălăgeanu A,** Sarafoleanu C, "Impact of smoking on immunological profile of patients with laryngeal carcinoma", *J Med Life*, 2009, II(2), 211-218, ISSN 1844-122x, PMID: 20108543 [PubMed - indexed for MEDLINE]

- **Lerescu L., Tucureanu C.**, Neagu S., Costea R., Gangura G, **Boghean Raluca,** Iana G., **Caras Iuliana** and **Salageanu Aurora** - Cytokine secretion pattern of peripheral blood mononuclear cells isolated from colon cancer patients undergoing surgery, 5th Balkan Congress of Immunology, June 01-03, 2008, Ohrid, Macedonia, Macedonian Journal of Medical Sciences 2008; 1(Suppl 1):S11-S22. ISSN 1857-5749
- **Tucureanu C., Lerescu L.,**Neagu S., Costea R., Gangura G, **Boghean Raluca**, Iana G., **Caras Iuliana** and **Salageanu Aurora** - Systemic cytokine and chemokine profile in colon cancer patients undergoing surgery, 5th Balkan Congress of Immunology, June 01-03, 2008, Ohrid, Macedonia, Macedonian Journal of Medical Sciences 2008; 1(Suppl 1):S11-S22. ISSN 1857-5749

- Ţucureanu C., Caraş I, Lerescu L, Melinceanu L, Pitica R, Costea R., Gangură G, Neagu S , Sarafoleanu C, **Salageanu A** "Perioperative immune profile in cancer patients", The 6th Balkan Congress of Immunology of BAIS, 28 April – 1 May 2010, Sibiu, Romania, Volum rezumate, pag 26, ISBN 978-973-88744-3-5

- **Sălăgeanu A, Tucureanu C, Lerescu L, Caraş I, Pitica R**, Gangurà G, Costea R, Neagu S. "Serum levels of adipokines resistin and leptin in patients with colon cancer" J Med Life, 3(4), 416-20, 2010

- Mincu-Radulescu G., L. Melinceanu, C. Sarafoleanu, **L. Lerescu, C. Tucureanu, A. Salageanu**, "Impact of smoking on immunological profile of patients having laryngeal cancer", XIX World Congress Of Oto-Rhino-Laryngology, June 1- 5, 2009 - São Paulo - Brazil, Abstracts Book

Projects:

Project PNII 41-004: Interventional strategies for the modulation of inflammation and immunosuppression in tumor progression: experimental studies and clinical correlations

II. Immunoadjuvants and immunomodulators

- **Immunologic characterization of micro- and nanostructured polysaccharide derivatives, with potential use as adjuvants**

The aims of the research were: (1) to evaluate amphiphilic, self-organizing polysaccharide derivatives with regards to their adjuvant potential and to shed light onto their mode of action;

One derivative, palmitoyl-carboxymethyl-pullulan (PCP), was shown to enhance antibody production, decrease the IgG1/IgG2a ratio and induce antigen specific IFN-gamma secreting spleen cells in mice having been immunized with influenza virus antigens combined with PCP as compared to antigen alone. PCP was further shown to greatly enhance immunogenicity of bacterial expressed influenza virus haemagglutinin (HA) and enhance cellular delivery of a fusion protein between GFP and the transmembrane domain of influenza HA by a cytochalasin and temperature dependent mechanism.

- **Bacterial immunomodulators: mechanisms of action, role of innate immunity**

The aims of research were: (1) to examine the mechanism by which bacterial immunomodulator CANTASTIM induces production of inflammatory cytokines in monocytes/macrophages; (2) to investigate the non specific protective effect of *Candida albicans* DNA in experimental infections.

Proinflammatory cytokines (TNF-alpha, IL-6) were induced in PMA-differentiated THP-1 cells by stimulation with ultra pure *E.coli* lipopolysaccharide (TLR4 agonist), synthetic triacyl lipopeptide Pam3CSK4 (TLR2 agonist) and CANTASTIM in the presence or absence of anti-TLR blocking antibodies or isotype matched control antibodies. Cells exposed to CANTASTIM produced significant levels of pro-inflammatory cytokines but the levels were lower then LPS-stimulated cells. Production of both cytokines was inhibited by treatment with anti-TLR2 blocking antibody and not by anti-TLR4 antibody. Also, silencing of TLR2 led to a statistically significant inhibition of TNF-a secretion induced by CANTASTIM while silencing of TLR4 had no effect on the response to CANTASTIM. These results support the hypothesis that CANTASTIM may exert its immunomodulatory and adjuvant activities through interaction of its bacterial components with TLR2.

Beside the protective effect of *Candida* DNA against systemic and gastrointestinal *C.albicans* infections, we demonstrated the prophylactic activity of ds DNA in adult mice against *Salmonella thyphimurium* infection, resulting in increased survival and tendency to reduce organ contamination. The results suggest that during acute systemic *C. albicans* infection, DNA released from the ingested pathogen could amplify an innate immune

response and could augment the production of pro-inflammatory cytokines. (Collaborative project in the Institute Pasteur Network, performed in collaboration with Pasteur Institute, Paris and Stephan Angelov Institute of Microbiology, Sofia, Bulgaria)

- **Immunomodulatory properties (anti-tumoral and/or anti-inflammatory) of natural (vegetal) extracts:**

The aims of research were: (1) development of *in vitro* and *in vivo* experimental models (cellular or non-cellular systems), able to supply information concerning immunomodulatory properties (anti-tumoral and/or anti-inflammatory) of natural extracts (especially vegetal extracts) or highly purified compounds isolated from natural extracts. Our studies are focused on the analysed products involvement in biological activities such as: modulation of efflux pumps (PgP, MRP) expressed on normal and tumoral cells, inhibition of enzymes responsible for arachidonic acid metabolism (COX-2, 5-LO activity inhibition, as well as mRNA COX-2 gene expression), modulation of some cellular markers (PI3K-γ, PPAR-γ and δ, PKB/Akt, NF-kB.

Several natural extracts have been purified and studies regarding their antioxidant capacity and also their potential to inhibit oxygen reactive species and nitric oxide have been carried out. Using notable chromatographic methods, Donatur GmbH partner (partner from Germany) obtained and purified natural extracts that have been tested with focus on their biologic activity. Six of these extracts have been investigated and we succeeded to identify one extract with oxygen radicals scavenger activity and three extracts with immunomodulatory properties. **(Study performed in collaboration with Donatur GmbH, Germany)**

ISI Publications:

- Remichkova M., Danova S., **Tucureanu C., Lerescu L., Salageanu A**., Dimitrova P., "Effect of Candida albicans dsDNA in Gastrointestinal Candida Infection", *Mycopathologia* 2009, 167(6), 333-340, ISSN 0301-486X
- **N.S. APETREI, A.-R. LUPU, A. CALUGARU**, F. KEREK, **G. SZEGLI, L. CREMER**, The antioxidant effects of some progressively purified fractions from Helleborus purpurascens acceptata spre publicare - Romanian Biotechnological Letters, Vol. 16, No. 6, 2011
- **L. CREMER, A-R. LUPU, M.M. BADULESCU**, G. MOCANU, D. MIHAI, **A. CALUGARU, N.S. APETRE**I, M. MOSCOVICI, **G. SZEGLI, „**Assessment of two synthesized curdlan derivatives as possible antioxidants and/or modulators of human PMN cells respiratory burst",, Romanian.Biotechnol.Lett.; 15(6), pag.5718-5728 (2010)

Other Relevant Publications:

- **Caras I, Tucureanu C, Lerescu L, Salageanu A,** „Bacterial Product Cantastim Derived from *Pseudomonas aeruginosa* Induces Migration and Maturation of Dendritic Cells", *Roum Arch*

Microbiol Immunol. 2007, 66 (1-2), 5-9, ISSN 1222-3891, PMID: 18928056 [PubMed - indexed for MEDLINE]

- **Anghelache I F, Caras I, Salageanu A**, „Adjuvant Properties of Bacterial Product Cantastim", *Roum Arch Microbiol Immunol*. 2007, 66(1-2), 17-21, ISSN 1222-3891, PMID: 18928058 [PubMed - indexed for MEDLINE]
- **Caraş I, Tucureanu C, Pitica R, Sălăgeanu A**. "Bacterial extract Cantastim activates macrophages via TLR-2", Roum Arch Microbiol Immunol., 70(1), 28-36,2011
- **Tucureanu C, Caras I, Lerescu L, Salageanu A, Onu A**, "Efficacy of New Formulations of Influenza Virus Antigens and Polysaccharide Derivatives in Inducing a Th1 Type of Immune Response", 2nd European Congress of Immunology, September 13-16, Berlin, Eur J Immunology, 2009, Vol. 39(S1), Abstract PD04/9, pag. S265, ISSN: 0014-2980
- **Pitica R, Caraş I, Ţucureanu C., Lerescu L, Salageanu A** "Human monocytic leukemia cell line THP-1 as a model for studying activation of Toll-like receptors" The 6th Balkan Congress of Immunology of BAIS, 28 April – 1 May 2010, Sibiu, Romania, Volum rezumate, pag 9, ISBN 978-973-88744-3-5
- **Caraş I, Ţucureanu C., Pitica R, Lerescu L, Onu A., Salageanu A** "Cantastim activates human macrophages by interaction with Toll-like receptor 2"The 6th Balkan Congress of Immunology of BAIS, 28 April – 1 May 2010, Sibiu, Romania, Volum rezumate, pag 9, ISBN 978-973-88744-3-5
- F.Kerek, A.Babes, **Lidia Cremer**, **Andreea-Roxana Lupu, C.Coman, Ana Calugaru, G.Szegli.** Local analgesic drug "MCS-18" provides high survival rates in septic-shock mice. *Shock*, 29 (Supplement 1), 110, 2008
- **Ana Calugaru, Lidia Cremer, Andreea-Roxana Lupu,** Maria-Mihaela Badulescu, Natalia-Simona Barzu, Franz Kerek, **Geza Szegli.** Recognition and control of Dectin-1 receptors and TLR-2 by Curdlan –like biopolymers and purified natural extracts. European Journal of Immunology, 39, S1, S77, 2009
- **Maria-Mihaela Badulescu, Natalia Simona Apetrei, Andreea-Roxana Lupu, Lidia Cremer, G.Szegli**, M.Moscovici, Georgeta Mocanu, Doina Mihai, Ana Calugaru, CURDLAN DERIVATIVES ABLE TO ENHANCE CYTOSTATIC DRUGS ACTIVITY ON TUMORAL CELLS, Roum.Arch.Microbiol.Immunol., Vol.68, Nr.4, pag.11-16, (2009)
- **Andreea-Roxana Lupu, Lidia Cremer, Geza Szegli, Ana Calugaru,** Maria-Mihaela Badulescu, Natalia-Simona Barzu, Maria-Luisa Flonta, Franz Kerek. NOVEL ANTIRHEUMATIC DRUG "MCS-18" INVOLVED IN TLR SIGNALING. European Journal of Immunology, Vol.39, No.S1, S77, 2009
- **Andreea-Roxana Lupu, Lidia Cremer**, F Kerek, **Ana Calugaru,** Maria-Mihaela Badulescu, Maria-Luiza Flonta, **G Szegli.** New natural compound MCS-18, a TLR-2 antagonist able to down-regulate inflammation-related pain. *European Journal of Pain*, 13, Suppl 1, 2009

- **Natalia Simona Apetrei, Ana Calugaru, Maria Mihaela Badulescu, Andreea Roxana Lupu**, M. Moscovici, Georgeta Mocanu, Doina Mihai, **G. Szegli, Lidia Cremer**, „The effect of some curdlan derivatives on Dectin-1 expression and cytokine production in human peripheral blood mononuclear cells", Roum.Arch.Microbiol.Immunol., Vol.69, Nr.2, pag.61-66, (2010)
- **Andreea Roxana Lupu**, Nicolae Georgescu,„Cold atmospheric plasma jet effects on V79-4 cells", Roum.Arch.Microbiol.Immunol., Vol.69, Nr.2, pag.67-74, (2010)
- F.Kerek, **G.Szegli, Lidia Cremer, Andreea-Roxana Lupu, Steliana Durbaca, Ana Calugaru, Aurora Herold, D.L. Radu,** The novel arthritis drug substance MCS-18 attenuates the antibody production in vivo, *Acta Microbiologica et Immunologica Hungarica*, 55 (1), 15-31, (2008)
- Bârzu Natalia Simona, Bădulescu Maria Mihaela, Lupu Andreea Roxana, Cremer Lidia, G.Szegli, F.Kerek, Călugăru Ana, Study of apoptosis induced by cytostatics and vegetal extracts on human endothelial cell line, R*oum.Arch.Microbiol.Immunol*., Vol.67, Nr.1-2, pag.5-9 (2008)
- **Maria-Mihaela Bădulescu, Andreea-Roxana Lupu, Lidia Cremer, Ana Călugăru, Natalia Simona Apetrei,** M. Moscovici, Georgeta Mocanu, G. Szegli, 16. The modulation of reactive oxygen species production from human polymorphonuclear cells by curdlan derivates as dectin–1 agonists/antagonists, *Roum.Arch.Microbiol.Immunol*., Vol.68, Nr.2, pag.63-68 (2009)

Projects:

- Biosynthesis, chemical modification , analytical and immunologic characterization of micro- and nanostructured polysaccharide derivatives, with potential use as adjuvants for new generation vaccines (F15)
- **Project CEEX M4 „***Development / modernization of the infrastructure of Immunomodulation Laboratory, Advanced Studies Department in National Institute of Research & Development for Microbiology and Immunology "Cantacuzino" – Bucharest, Romania, for integration, validation and accreditation of modern methodologies for testing the efficiency of drugs recommended in the therapy of chronic and acute inflammatory diseases",*financed by National Authority for Scientific Research.
- **Project PN II/2007** (Biotehnology) „*New microorganisms capable of enzymatic synthesis of therapeutically active biopolymers, using glycerin (by-product of biodiesel obtaining) as substrate*
- ***Collaborative project with Donature SRL, Timisoara:*** *"Collaborative project for a preliminary research concerning some biological activities of MCS-18 and other plant extracts"*

- ***Project PN II/2008*** *(Biotehnology) „NEW NATURAL COMPOUNDS INVOLVED IN MODULATION OF HMGB-1 ACTIVITY, A TARGET PRO-INFLAMMATORY CYTOKINE IN SEPTIC SHOCK TREATMENT AND IN NEOPLASTIC DISEASE THERAPY„*

OTHER STUDIES

Study of biocompatibility/cytotoxicity of different materials

The aims of research were to evaluate the biocompatibility/citotoxity of different materials (e.g. polymers used for bone implants, organometallic compounds etc) using cytotoxicity tests on mouse fibroblast cell line L929. The cell monolayers were grown to near confluence in flasks and then exposed to test biomaterials directly or indirectly by means of fluid extracts. The cell viability was measured using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. **(Study performed in collaboration with Politehnica University, Bucharest)**

ISI Publications:

- Zecheru T., Zaharia C., **Sălăgeanu A.**, **Ţucureanu C**., Rusen E., Mărculescu B., Rotariu T., Cincu C., "Polymeric biocompatible structures for controlled drug release", *J Optoelectr Adv Mat.* 2007, 9 (9), 2917 - 2920, ISSN 1454-4164

- Zaharia C., Zecheru T., Rusen E., **Sălăgeanu A.**, Cincu C., „Methylmethacrylate-Iodothiophene copolymers for the obtaining of bone and dental cements", *J Optoelectr Adv Mat.* 2007, 9 (11), 3307 - 3311, ISSN 1454-4164

Other Relevant Publications:

33. Niculescu V-C, Muresan N, Salageanu A, Tucureanu C, Marinescu G, Chirigiu L, Lepadatu C, "Novel 2,3-disubstituted 1,4-naphthoquinone derivatives and their metal complexes – synthesis and in vitro cytotoxic effect against mouse fibrosarcoma L929 cells", J Organomet Chem, doi:10.1016/j.jorganchem.2011.10.036, 2011(online)

Projects:

CEEX 11/2007: New micro and nanostructured composites with application in construction, bioenergy and food safety (F1)

CEEX 2008: Performance materials based on chemically modified and / or nanostructured polymers for industrial and medical applications (F21)

T10 Cellular Immunology /Immunopathology

Team leader: Dorel Lucian Radu

Research members:

Dorel Lucian Radu	MD, PhD, Senior Researcher, General Director	CS1
Matache Cristiana	PhD, Senior Researcher (until 2010)	CS1
Stefanescu Maria	PhD, Senior Researcher (until 2009)	CS1
Pistol Gina	PhD, Researcher (until 2010)	CS3
Besliu Alina	Researcher (until 2010)	CS3
Adina Daniela Iancu	Researcher, Head of Laboratory	CS3
Mihai Penescu	veterinary physician	NRDS
Liliana Popister	Laboratory technician	NRDS
Radu Iulian Tanasa	veterinary physician, PhD, Senior Researcher	CS2
Lixandra RUSEANU	Laboratory technician	NRDS
Henriette Stavri	PhD, Senior Researcher	CS1
Ulea Irina	Researcher	CS
Crina Stavaru	MD, PhD, Senior Researcher, Head of Laboratory	CS2
Leontina Banica	Researcher	CS3
Orhan Rasid	Technician	T1
Daniela Florescu	Laboratory technician	NRDS
Doina Proteasa	Laboratory technician	NRDS

Main fields of research:

Autoimmunity

Main research topics:

Studies regarding cellular and molecular defects involved in etiopathology of connective tissue diseases

The aims of research were: (1) to decipher the mechanisms involved in peripheral tolerance; (2) to establish the contribution of regulatory T cells in the development and maintenance of connective-tissue diseases; (3) to elucidate the role of some molecules in the development and maintenance of autoimmunity and tissue/organ dysfunctions in Systemic Sclerosis; (4) elaboration of experimental models for the study of connective-tissue diseases; (5) identification of new therapeutic target.

Studies performed on patients with Systemic Sclerosis (SSc) in order to establish the possible roles of Semaphorin 4D (CD100) and CD72 molecules in etiopathogeny of SSc lead to important observations. Augmented percentages of $CD100^{high}CD4^{+}$ and $CD100^{high}CD8^{+}$ T cells and increased serum sCD100 levels were identified in SSc patients in contrast with healthy donors (HDs). When compared SSc with Systemic Lupus Erythematosus (SLE) patients some differences were also identified. $CD100^{high}CD3^{+}$ and $CD100^{high}CD8^{+}$ T, $CD100^{high}CD19^{+}$B cell percentages and serum sCD100 levels significantly differentiated between SSc and SLE patients. SSc B cells expressed an augmented level of CD19 than HDs B cells. CD100 dysregulations were directly correlated with anti-Scl70 antibodies production, interstitial lung disease or active inflammation while $CD19^{high}$ and $CD19^{+}CD72^{+}$ B cell percentages were correlated with thickening of skin quantified by modified Rodnan skin score or anti-centromere antibodies, respectively. High percentage of $CD100^{high}$ positive cells and increased level of sCD100 observed by us in SSc patients are not common characteristics for all autoimmune diseases (such as SLE). The high level of sCD100 in SSc patient sera seemed not to be an epiphenomenon of pro-inflammatory micro-environment in SSc patients since 80% from SLE patients also presented positive values of inflammatory markers (ESR, CRP) without having high level of serum sCD100. (Study performed in collaboration with “Sf. Maria” Hospital)

The ability to actively suppress an immune response makes regulatory T cells (Tregs) an attractive candidate as a novel therapeutic agent for treating autoimmune diseases, allergy and transplant rejection. In our experiments on *in vitro* expansion of Tregs two drugs recognized for their capacity to reestablish peripheral tolerance (Rapamycin) or for immunomodulatory activity {1,25-Dihydroxyvitamin D(3) [1,25(OH)(2)D(3)], the biologically active metabolite of Vitamin D3} were used. Our results showed that: (1) *in vitro* expansion of Tregs is dependent on antigenic $CD4^{+}$T cells stimulation and independent of the manner to deliver the stimulus; (2) both Rapamycin and 1,25(OH)(2)D(3), after 14 days of culture determine the increase of $CD4^{+}CD25^{high}Foxp3^{+}$T cells frequency in $CD4^{+}$T cells population; (3) as compared with Rapamycin,

1,25(OH)(2)D(3) has a lower efficiency to expand Treg, but its presence during mixed leukocyte reaction increases the suppressive ability of Rapa-expanded Tregs.

ISI Publications:

- Besliu A, Banica L, Predeteanu D, Vlad V, Ionescu R, Pistol G, Opris D, Berghea F, Stefanescu M, Matache C. Peripheral blood lymphocytes analysis detects CD100/SEMA4D alteration in systemic sclerosis patients. Autoimmunity. 2011 Aug;44(5):427-36. doi: 10.3109/08916934.2010.541171. Epub 2011 Jan 19.

- Banica L, Besliu A, Pistol G, Stavaru C, Ionescu R, Forsea AM, Tanaseanu C, Dumitrache S, Otelea D, Tamsulea I, Tanaseanu S, Chitonu C, Paraschiv S, Balteanu M, Stefanescu M, Matache C. Quantification and molecular characterization of regulatory T cells in connective tissue diseases. Autoimmunity. 2009 Jan;42(1):41-9.

- Pistol G, Matache C, Calugaru A, Stavaru C, Tanaseanu S, Ionescu R, Dumitrache S, Stefanescu M. Roles of CD147 on T lymphocytes activation and MMP-9 secretion in systemic lupus erythematosus. J Cell Mol Med. 2007 Mar-Apr;11(2):339-48.

- Lemke H, Tanasa RI, Trad A, Lange H, 2009. Benefits and burden of the maternally-mediated immunological imprinting. Autoimmunity Reviews 8: 394-399.

Other relevant publications:

- Beşliu AN, Pistol G, Marica CM, Bănică LM, Chiţonu C, Ionescu R, Tănăseanu C, Tamsulea I, Matache C, Stefănescu M. PI3K/Akt signaling in peripheral T lymphocytes from systemic lupus erythematosus patients. Roum Arch Microbiol Immunol. 2009 Apr-Jun;68(2):69-79.

- Beşliu AN, Bănică LM, Lonescu R, Predeţeanu D, Stăvaru C, Marica CM, Chiţonu C, Pistol G, Stefănescu M, Matache C. Role of cellular immunity in systemic sclerosis pathogenesis: update on CD4+T cells population studies. Roum Arch Microbiol Immunol. 2009 Jan-Mar;68(1):5-13.

- D. Opris, A. Besliu, R. Ionescu, D. Predeteanu, L. Banica, C. Chitonu, G. Pistol, M. Stefanescu, C. Matache Semaphorin 4D involvement in Systemic Sclerosis,, *1st Systemic World Congress*, february 11-13, 2010, Florence, Italy, *Clinical and Experimental Rheumatology*, 2010, abstract SP.01.33, pg 35

- Semaphorin 4D And its Receptor CD72 Mediate some Abnormalities in Human Autoimmune Diseases, Alina Besliu, Leontina Banica, Gina Pistol, Denisa Predeteanu, Ruxandra Ionescu, Violeta Vlad, Maria Stefanescu si Cristiana Matache, 28 April – 1 May 2010, Sibiu, ROMANIA, The 6th Balkan Congress of Immunology of BAIS.; The 40th National Conference of RSI; 29 April Day of Immunology; The 19th National Conference of SRAIC;

Projects:

- Advanced research in immune tolerance in order to identify novel therapeutic pathways in the pathology of connective tissue vascular diseases (F6)
- Studies on signaling through pi3k/akt/mTOR in peripheral blood T lymphocytes from SLE patients aimed at identifying new therapeutic target (F27)
- Genomics and proteomics research in systemic sclerosis pathology in order to open new therapeutic perspectives (F35)

Experimental studies using a murine model of type 1 diabetes

The aims of research were: (1) The investigation of cellular and molecular mechanisms involved in type 1 diabetes mellitus in a double transgenic mouse model; (2) Exploration and evaluation of complications (metabolic, somatic morphopatological modifications) induced by type 1 diabetes using an animal model and different therapeutically modalities; (3) A preliminary study of diabetic neuropathy using an experimental murine model. (Study performed in collaboration with Faculty of Biology, University of Bucharest)

An alternative experimental approach for the study of type 1 diabetes is the utilization of INS-HA+/−,TCR-HA+/− double transgenic (dTg) mice, expressing the hemagglutinin (HA) of PR8 virus in pancreatic β-cells (INS-HA) and at the same time the T-cell receptor (TCR-HA) specific for the immunodominant CD4 T-cell epitope of HA ($HA_{110–120}$). Our results revealed that double transgenic mice (dTg) presented high levels of blood glucose levels (>200 mg/dL) as compared to normal mice and glucosuria correlated to low levels of insulinemia. Also, dTg body weight was lower in comparison to non-diabetic mice. *In vitro* evaluation of reactive oxygen species (ROS) production showed an showed an increase in oxidative activity as well as in nitric oxide (NO) production in polymorphonuclear (PMN) cells and in peritoneal macrophages harvested from dTg mice as compared to normal ones. These results indicate that beside the autoimmune component, oxidative stress represents an additional factor in the development of diabetes complications. Immune cell phenotyping revealed that the percentage of CD45R/B220+ and NK1.1+ cells was higher and the percentage of CD4+ and CD8+ lower in both peripheral blood and spleen of dTg mice as compared to control mice.

One of the major complication of type 1 diabetes is represented by diabetic neuropathy. The loss of peripheral sensory neurons that accompanies diabetic neuropathy and its etiology are not yet elucidated. We have tested the effect of intraperitoneal administration of capsaicin on reactive species damage. Capsaicin administration in diabetic mice led to the reduction of NO production by peritoneal macrophages, without significant changes in ROS production in PMN cells. The results highlighted changes in the action potentials generated by sensory neurons from diabetic mice; these changes were attenuated or even abolished by capsaicin administration. All the

experiments led to the conclusion that dTg mice represent a suitable animal model to study type 1 diabetes and further diabetic neuropathy.

ISI Publications:

- Uyy E, Antohe F, Ivan L, Haraba R, **Radu DL**, Simionescu M. Upregulation of caveolin-1 expression is associated with structural modifications of endothelial cells in diabetic lung. *Microvasc Res.* 2010 Mar;79(2)154-9.

Other relevant publications:

- Marinescu B, Coman C, **Iancu AD, Stavaru C,** Lupulescu E, Onu A, **Radu DL.** Evaluation of the efficacy of a specific hyperimmune serum in experimental influenza infection in mice. *Roum.Arch.Microbiol.Immunol,* 2009 Apr-Jun;68(2):80-2
- Radu BM, Radu M, **Iancu AD**, Rotaru D. Effect of GppNHp on GIRK currents in dorsal raphe nucleus neurons from 5HTT–/– mice. *Romanian J. Biophys*., 2009, 19(3): 159–170.
- Radu BM, **Iancu AD**, Marin A, Radu M., Banciu D.D., **Stavaru C, Radu D.L**.. Basic features of sensory neurons from dorsal root ganglia in TCR-HA+/–/RIP-HA+/– MICE**.** *Romanian J. Biophys*., 2009, 19(2): 83–95.
- **Grosu M., Stavaru C.**, Guta D, **DL Radu.** Pancreatic beta cell allotransplant in double transgenic mouse model of type 1 diabetes mellitus. *Roum Arch Microbiol Immunol.*, 2007, 66(3-4):57-61, ISSN: 1222-3891
- Radu BM, Marin A, Rotaru DI, **Iancu AD**, Mustaciosu C, **Radu DL**, Flonta ML, Radu M, , Imaging the methylglyoxal-induced changes in the peripheral sensory neurons from dorsal root ganglia, *12th Conference on Methods and Applications of Fluorescence (MAF 12) Spectroscopy, Imaging and Probes*, 2011, France, Strasbourg.
- Radu BM, Rotaru D, **Iancu AD, Radu DL**, Radu M., Methylglyoxal effect on sensory neurons from dorsal root ganglia DRG in type 1 diabetes mice, *EMBO|EMBL Symposia: Seeing is Believing - Imaging the Processes of Life*, 2011, Germany, Heidelberg
- Radu BM, **Iancu AD, Radu DL, Radu** M., Is double transgenic mice TCR-HA+/-/Ins-HA+/- a proper model for peripheral diabetic neuropathy? *50th Annual Meeting of the American Society of Cell Biology*, 2011, Pennsylvania, Philadelphia
- Radu BM**, Iancu AD, Radu DL,** Radu M., Capsaicin decreases blood glucose, insulin and TRPV1 expression in sensory peripheral neurons in double transgenic diabetes mice, 1st International Diabetes and Obesity Forum, 2010, Greece, Athens, Diab Obes Metab J 12(S1): 41

- **Iancu AD,** Radu BM, Radu M, **Stavaru C, Radu DL,** Double transgenic mice with type I diabetes - a model of diabetic neuropathy, *Joint Meeting of Immunology and Clinical Allergology*, 2010, Romania, Sibiu

- Radu B.M., **Iancu A.D.,** Rotaru D., **Radu D.L., Stavaru C.,** Mustaciosu C., Radu M., Capsaicin is a key molecule in neuropathic pain induced by diabetes, *35th FEBS Congress*, 2010, Sweden, Goteborg, FEBS J, 277(S1):77

- **Iancu A.D., Stavaru C.,** Petcu I., Radu B.M., **Radu D.L.,** Radu M., , Oxidative stress alterations in peritoneal macrophages and polymorphonuclear leukocytes for type I autoimmune diabetic mice, *35th FEBS Congress,* 2010, Sweden, Goteborg, FEBS J, 277(S1):67

- Radu B.M., **Iancu A.D., Radu D.L.,** Radu M. TRPV1 role in diabetic neuropathy evaluated in a double transgenic diabetic mice, *7th FENS Forum of European Neuroscience*, 2010, The Netherlands, Amsterdam

- Radu B.M., Radu M., **Iancu A.D., Stavaru C., Radu D.L.** Changes in the level of expression for voltage-gated sodium channels and acid sensing ion channels induced by genetic diabetes, *XXth International Symposium on Bioelectrochemistry and Bioenergetics*, 2009, Romania, Sibiu
- **Iancu A.D.,** Dinu C., Neagu M., **Radu D.L.** Immune cell phenotyping after Salmonella typhymurium infection in murine experimental models. *„2nd European Congress of Immunology"*, 2009, Germany, Berlin

- **Stavaru C., Iancu A.D.,** Constantin C., Neagu M., **Radu D.L.**, Ion R.M., , "THE *IN VITRO* PHOTODYNAMIC EFFECT OF FUNCTIONALIZED FULLERENS ON HUMAN LEUKOCYTES" *„2nd European Congress of Immunology"*, 2009, Germany, Berlin

- Macri B.M., Marin A., **Iancu A.D., Stavaru C., Radu D.L.** Melittin effect on the sensory neurons prelevated from double transgenic vs. normal mice, *30th European Peptide Symposium*, 2008, Finland, Helsinki, J Pept. Sci. Suppl 14(8):148

- Macri B., **Iancu A., Stavaru C., Radu D.L**., Electrophysiological characteristics of sensitive neurons from double transgenic mice with type I diabetes compared with normal mice, 33rd FEBS Congress & 11th IUBMB Conference, 2008, Greece, Athens, FEBS J 275(S1):355

- Militaru M, **Stavaru C,** Soare T, Ciobotaru E, **Grosu M, Radu DL** - Cytological features of upper respiratory pathways in acute influenza infection in mice -experimental model"- *25th Annual Meeting of European Society of Veterinary Pathology*, 29 august-1 septembrie 2007, Germany, Munich.

Projects:

- Mechanisms of neuropathic pain in type I diabetes transgenic mice (F40)

Study of the immune response in experimental models of bacterial infection

The aim of research was to develop a novel immunoassay for the very early detection of biothreatening bacterial infection. The proposed test will quantify specific carboxy (C)-terminal peptides originating from prothymosin α (proTα) and released in serum, in order to detected amount of which will be linked to the onset and/or severity of the infection. (Study performed in a collaborative project funded by NATO SfP)

ISI Publications:

1. Stavri H, Ulea I, **Radu DL**, Branaru MG, Moldovan O, Bogdan MA, Tudose C, Raileanu M, Duiculescu D, Ene L, Olar V, Ionita C, Popa GL, Popa MI, Brennan PJ. Serodiagnosis of enviromental mycobacterial infections. *J Microbiol Methods. 2011 Sep;86(3):283-90*.

Other relevant publications:

- Ceausu M., Butur G, Ceausu Z, D. Radu, Ardeleanu C. Bacillus anthracis, an ancient bacterium in a modern warfare: bioterrorism. A biological and histopathological review. *Archives of the Balkan Medical Union,* 2007, 49(3): 182-185, ISSN: 0041-6940

Projects:

Development of a Novel Immunoassay for the Very Early Detection of Biothreatening Bacterial Infection (CF9).

OTHER STUDIES

Nanotechnology: *In vitro* studies of toxicological and pharmacological profile of fullerens compounds

(Study performed in collaboration with "Victor Babes" National Institute for Pathology)

Development of monoclonal antibodies

- Development of monoclonal antibodies (mabs) and immunoassays for detection of highly pathogenic avian influenza (HPAI) with A/H5N1 subtype. We have developed murine mabs against H5N1 influenza A viruses using the vaccine candidate strain NIBRG-14 (clade 1 background). We further analyzed five IgG mabs against HA that exhibited immunoreactivity

(by IHA, MNA, ELISA, WB) with different H5N1 strains from clades 1 and 2.2 – including HPNAI virus strains isolated in Romania, from birds, during the 2005-2006 outbreaks. Based on three of these mabs we have developed an antigen-ELISA (Ag-ELISA) for detection of influenza virus H5 subtype. This assay is currently in process of validation.

- Development of monoclonal antibodies (mabs) against *Leptospira interrogans serovar icterohaemorrhagiae* and *Leptospira interrogans serovar wolffi.* Since during the lasts years Romania has reported a high prevalence of leptospirosis in humans (200-500 cases/year), which means an overall notification rate of 0.49-2.09 per 100,000 population, we have prepared mabs against those 2 mentioned serovars responsible for the majority of human cases. Our laboratory could isolate 3-4 clones which specifically reacted in ELISA and microagglutination test (MAT), and we are currently further characterizing these mabs for developing diagnostic tests that may be an efficient alternative to MAT-which needs maintaining of living reference cultures of leptospires in dedicated laboratories.

ISI Publications:

Constantin C, Neagu M, Ion RM, Gherghiceanu M, Stavaru C. Fullerene-porphyrin nanostructures in photodynamic therapy. *Nanomedicine (Lond).* 2010 Feb;5(2):307-17. Review.

Other relevant publications:

34. Rodica Mariana Ion, Radu Claudiu Fierascu, Monica Neagu, Carolina Constantin, and Crina Stavaru. Porphyrin (TPP)–Polyvinylpyrrolidone (PVP)–Fullerene (C60) Triad as Novel Sensitizer in Photodynamic Therapy. Science of Advanced Materials Vol. 2, 223–229, 2010. ISSN: 1947-2935

T11_Biotechnology and vaccine development

Head: Adrian **Onu** Sci/MD, PhD, senior researcher (CS 1), Adrian.Onu@cantacuzino.ro

Deputy: Dorel Lucian **Radu** MD, PhD, senior researcher (CS 1), Dorel.Radu@cantacuzino.ro

Laboratory members:

Nadia **Bucurenci** (retired 2011) – Sci , senior researcher (CS I),

Adrian **Onu** (1.00) – Sci, PhD, senior researcher (CS I)

Dorel Lucian **Radu** (0.5) MD, PhD, senior researcher (CS1)

Adriana **Costache** (1.00) – **-**Sci , senior researcher (CS III)

Leontina **Banica (**0.5) – Sci **,** senior researcher (CS III)

Daniel **Chirita (**0.25) – Sci **,** assistent researcher (CS III)

Aurora **Salageanu** (0.25) –Sci, PhD, senior researcher (CS I)

Catalin **Tucureanu** (0.25) –Sci/MD, PhD, senior researcher (CS)

Iuliana **Caras** (0.25) – Sci , senior researcher (CS III)

Vlad Constantin **Tofan** (0.25) – **,** Sci, asistent researcher (AC)

Emilia **Lupulescu** (0.25) – Sci , senior researcher (CS III)

Alina **Baietel Ivanciuc** (0.25) – **,** Sci, researcher (AC)

Marilena **Mihai** (0.25) – Sci , senior researcher (CS III)

Laurentiu **Berbecila** (0.50) –Sci/MD, researcher (CS)

Alina **Ghiorgisor** (0.50) – Sci , researcher (CS)

Andreea **Savu** (0.50) – **,** Sci, asistent researcher (AC)

Mariana **Saulea (**0.50) – Sci **,** senior researcher (CS III)

Alina **Mihalcea (**1) – Sci **,** senior researcher (CS III)

Mariana **Lixandru** (0.50) –Sci, researcher (CSIII)

Adreea **Zaharie** (0.50) – Sci , assistant researcher (CS)

Francisc **Czobor (**0.5) – Sci **,** senior researcher (CS III),

Adrian **Cotro (**0.50) – Sci **,** senior researcher (CS)

Andreea Busa (0.25)-researcher (CS)

Claudia Ursu (0.25)-researcher (CS)

Gabriela Gherganu (0.25)-researcher (CS)

Anca Ciobotaru (0.25)-researcher (CS)

Geirgeta Militaru (0.25)-research assistant (AS)

Mission, objectives, research interest

The team has as main objective the development of the new technology in vaccine production covering may aspect from protein expression and design to downstream processing. In order to implement these methods in practice a complex team was created that is developing **research collaborative projects** and is conducting and befits from **training activities.**

Objectives of our activity are consisting in:

- Modern vaccine development, the legacy of the Cantacuzino Institute, with a special focus to emergency one.
- Developing competences in current biotechnology from fermentation to downstream processing and process analytical technologies.
- Improving capacities in protein cloning and expression ;
- Developing protein based technology from classical purification to modern proteomics.

Research activity

I.VACCINE DEVELOPMENT

Vaccine development is the legacy of the Cantacuzino Institute and covers a complex range of activities covering different fields of biomedical R&D from microbiology and immunology to biotechnology and pharmacology. This activity benefit the most from the concept of integrating public health / research and development / production which was originally demonstrated in vaccination campaigns in 1913 and 1916 when during the Balkan war. Thus, based on scientific observations Professor Cantacuzino team succeed to vaccination an entire army corps (50,000 men) and to stop the epidemic, success that contributed the creation of Cantacuzino Institute. Due to the complexity this meta-project was the result of several smaller project and grants. Since then there was a continuous effort to update the technology in different vaccine. The latest example is the pandemic vaccine development where the formal activities started in 2006 with two projects, one, internal **"Experimental model of influenza virus strains of immunological research and clinical studies of human infection to establish a methodology for evaluation of influenza vaccines"** - Acronym: EVAGRIP CEEX 160 / 2006 (2006 – 2008) and the other, external "**Combating flu in a combined action between the industry and the public sector in order to secure adequate and fast intervention in Europe**" FluSecure, a DG-SANCO. The high amount of resources needed to build capacities were obtained from other external grants: **WHO influenza vaccine production capacity building grant** (Letter of Agreement SPHQ09-LDA-86), was dedicated not only for technology but also for developing research infrastructure. The grant was completed by an **adjuvanted influenza vaccine development grant** with Infectious Disease Research Institute, Columbia Street, Suite 400 Seattle, WA 98103 (F16SUB-2010), subsidiary of the main grant 1 IDSEP100008-01-00 1124 funded by de ASPR/BARDA/AMS, „**Development and Sustainable Manufacturing of Adjuvanted Pandemic Influenza Vaccines in Developing Countries**".

There were other smaller project as traditionally based on own resources like "**Redefining procedures of fragmentation for production of vaccine Influenza"** (PN 09220401 - CS Alina Ghiorghisor), or "**Development of technologies for vaccines production and conditioning based on disposables technologies"** (PN 09220402CS Laurentiu Berbecila) which moved towards a modern collaborative integrated research joining effort with EU lager projects as **FASTVAC: A generic framework forum FAST production and evaluation of emergency Vaccines (**DG SANCO / FASTVAC - CSI Dr. Dorel Lucian Radu) were our researchers had the opportunity to work together with intentional teams.

Another task was the development of a new type the vaccine adjuvants started from a collaborative project leaded by NI for Pharmaceutical and Chemical R&D - **Biosynthesis, chemical modifications analytical and immunological characterization micro-and nanostructured of polysaccharides used as adjuvants for the new generation of vaccines**(CEEX BIOTECH 66/2006 – IC representative CSI Dr.A.Onu). One of the potential adjuvant turns out to very promising and therefore the researches has been continued with another project **The study of nanostructured adjuvants usable for the new generation of vaccines** (PN 09220202 - CS Catalin Tucureanu). The "adjuvant team" created succeeded to transfer technology and further develop a new vaccine candidate in the U.S. Financed grant **Development and Sustainable Manufacturing of**

Adjuvanted Pandemic Influenza Vaccines in Developing Countries (ASPR / BARDA / AMS - CSI Dr. Adrian Onu).

The third lines of the development were the cell culture based technology development covered by projects which started to develop the use of modern technology in the field as "**Cellular Technologies for vaccines"** (CEEX BIOTECH 83 / 2006 - CSI Dr. Adrian Onu) that have been continued with other projects **Development of vaccines on base of cells for viruses influenza** (PN 09220301- CS Alina Baetel). Cell based vaccine in therapy has also been explored **Studies of the development T cells based vaccines as a new regulatory therapeutic way in autoimmune and allergic disease and for prevention rejection grafts (**PN 09220302 CSIII Leontina Banica)

II. FERMENTATION BIOTECHNOLOGY, ENZYMOLOGY AND APPLIED MICROBILOGY

The fermentation based technologies is very important to build a sustainable research in biotechnology. Therefore we heave focused our efforts in developing projects to improve bacterial fermentation knowledges as "**Advanced biotechnology methods in preparation biological products for therapeutic human use with integration of optimized methods for quality and of control in compliance with EU standards and rules** (CEEX BIOTECH 6 / 2005 - CSI Dr. Adrian Onu) continued by "**Advanced algorithms of multi-criteria decision and intelligent management of preparation of therapeutic biotechnology products for human use** (62-051 CSI - Dr. Adrian Onu – In a partnership leaded by Polytechnic University Bucharest. The yeast fermentation development project joined the Cantacuzino Institute antioxidant products tradition in "**Development of advanced biotechnological technologies for preparation of pharmaceutical products with antioxidant torularhodin and study potential therapeutic applications"** (61-021 CSI - Dr. Aurora Salageanu).

An interesting concept was the one of Enzymology and Applied Microbiology Laboratory created to secure and to improve the competence of research based on the genetic engineering technology and protein expression. It has a balanced activity between applied and fundamental research topics with projects such as "**Guanosine monophosphate kinases of pathogenic bacteria: factors of virulence and potential targets for the antibacterial drugs. (In the phase I "Cloning and overexpression of four GMP kinases of pathogenic bacteria ") (**PN 06150201-CSI Dr. Bucurenci Nadia) but also started to be involved in protein cloning for the new generation of products in the project "**Making a new test tuberculin cutaneous for revelation hypersensitivity of type delayed by technologies advanced with use antigens recombinant specific for diagnosis TB latent and active (**61-026 -CSI Dr. Henriette Stavri) or **Implementation of technologies for obtaining recombinant mammalians cytokines (**PN 09220303 -CS III Adriana Costache). Moreover the competences of the laboratory team in protein purification involved them in other biotechnology projects such as "**Implementation and optimization technological process for obtaining of therapeutic sera with component active F (ab)2' to of highly agents pathogens bacterial and viral" (** 61-019 - CSI Dr. Nadia Bucurenci).

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- *Bogdan Marinescu, Cristin Coman, Adina Daniela Iancu, Crina Stavaru, Emilia Lupulescu,* ***Adrian Onu****, Dorel Lucian Radu – Evaluation of the efficacy of a specific hyperimmune serum in experimental influenza infection in mice, Romanian Archives of Microbiology and Immunology, Volume 68 – No. 2, April-June* ***2009****.*

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- Radu D.L., Militaru M., Ionescu A., Stavaru C., Lupulescu E., **Onu A.**, Grosu M., Marinescu B., Oprisan G., Dimitriu R., **Costache A**., Onita I. "Studiul experimental si clinic al infectiei gripale si al imunizarii cu vaccinuri gripale", Ed. Universitara „Carol Davila" Bucuresti, 2008.

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different agents, National Institute for Research and Development in Microbiology and Immunology, Romania, ESWI Third European Influenza Conference 14-17 September, **2008**, Vilamoura, Portugal.

67. WHO influenza vaccine production capacity building grant Grant Agreement: SPHQ-LOA-86 / 24 June 2009 “Cantacuzino” National Institute for Research and Development in Microbiology and Immunology Bucharest – ROMANIA, Cancun, Mexic, Mai **2011**.

The continuous education of young researcher is the characteristic of the scientific policy of the team.

Laurentiu **Berbecila,** Alina **Ghiorgisor,** Mariana **Saulea**, Alina **Mihalcea,** Mariana **Lixandru ,** Adrian **Cotro** and Daniel **Chirita** received specific training for modern vaccine technology in the US.

T12_Experimental_pathology_and GLP preclinical testing unit

Head: Crina Stavaru **Stavaru,** MD, PhD, senior researcher (CS II),

Deputy: Coman **Cristin** VMD, PhD, senior researcher (CS II)

Laboratory members:

Dorel Lucian **Radu** 0.25

Adina Daniela **Iancu** 0.25

Daniel **Chirita** 0.75

Orhan Rasid 0.75

Daniel **Chirilita** 0.75

General objectives of our activity consist in: Methodological development for clinical testing of biological products in order to elaborate standard operating procedures conforming to national (ANM) and european (EMEA) guidelines.

Specific objectives

- Development of preclinical study plans for bacterial /viral derived vaccines and /or immune modulators
- Development of imunogenicity testing methodology for bacterial and viral vaccines: comparative tests of different protocols in order to discern optimum methods by:

70. Assessment of proper preclinical safety testing for bacterial and viral vaccines: single dose toxicity, multiple dose toxicity, tolerance testing;
71. Statistical analysis of results and endorsement of protocols by the Quality Control department

Research activity

The animal experiment is a tradition of the Cantacuzino Institute. There several facilities located in main campus and in Baneasa campus. Both underwent active development in the latest

years. In term of capacity building experimental laboratory animal work benefited from - EVAGRIP – project. . The project "Experimental model of influenza virus strains of immunological research and clinical studies of human infection to establish a methodology for evaluation of influenza vaccines" - Acronym: EVAGRIP CEEX 160 / 2006 (2006 – 2008) Coordinator: National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" Project Director: Associate Professor Dorel Lucian Radu with the collaboration of Institute for Diagnosis and Animal Health - Project Coordinator: Dr. Aurelia Ionescu, University of Agronomic Sciences and Veterinary Medicine Bucharest - Project Coordinator: Prof. Manuella Militaru . The project aimed to the assessment of influenza vaccines methodology that could be applied in preventing influenza, comparative study of methods of immunization by influenza vaccine administration in humans, experimental influenza infection in animal model including transgenic mice, histology of harvested organs. Based on this a study of influenza infection was published.

There were several outcomes from this project in different vaccine research fields. Regarding the animal experiment development , the main outcome was the opportunity to work with candidate pandemic vaccine strains (H5N1) and to verify and develop the requested techniques for immune response evaluation. A very important achievement was the establishment of a partnership with institutions with high competence in animal histopathology which was very important for the development of the project.

Baneasa campus is the main experiment region of the institute. Originaly a kind donation from the Royal family it was the region where the therapeutic sera were produced. When the demand of such sera diminished, the unit got involved little by little in modern animal experiment work. Modernisation started with the establishment of a SPF animal laboratory facility with research infrastructure supported by the National Research Agency. A CEEX Module IV project **Development / modernization infrastructure existing in Immunomodulation Laboratory of Center of Advanced Studies NIMRD Cantacuzino for the integration, validation and accreditation of modern methodology for testing the efficiency of drugs recommended in therapy inflammatory acute and chronic diseases** (209/2006 CSII Dr. Lidia Cremer) helped the unit to develop specific competence in the field. The project **"Upgrading of research infrastructure for growth, maintenance and use for scientific experiments and other laboratory animals Baneasa location, National Institute for Research Development in Microbiology and Immunology - BIOSCANT** Capacities Program - Modules I and II competition in 2008 –209/2006 CSII Dr. Cristin Coman) , highly improved the research infrastructure –and will become the location of the upcoming GLP unit.

The competence of the researchers from Banesa was recognised by the collaborative research projects based on experimentation on laboratory animals such as "**Research on development of techniques ELISA / RIA for detection contaminants chemical Food: trenbolone and nandrolone"** (CEEX BIOTECH 24/2005 - CSI Dr. Adrian Onu) or "**Research on development of technique ELISA for detection contaminant pesticides 3.6 dichloro-2-metoxibenzoic of samples Food and of the environment."** (51-086 - CSI Dr. Adrian Onu) both using in-house prepared immune sera.

The EU financed FLUSECURE project "**Combating FLU in a combined action between industry and the public sector in order to SECURE adequate and fast interventions in Europe**"

The work package 5, "**EFFECTIVE VACCINES"** had the task of augmenting the immunogenicity of the current influenza vaccine using an adjuvant system and to establish an optimal immunization strategy giving rise to long-lived B- and T-cell responses using a simple vaccination regime, involving therefore a consistent animal experimentation part. A major outcome from these tests was the development and validation of a microneutralization assay, currently used for the evaluation of immune response, which allowed later its use for nonclinical and clinical tests. Also participation is such a consortium increased our credibility, opening opportunities for other funds.

The best example was WHO influenza vaccine production capacity building grant 2009-2011 (Letter of Agreement SPHQ09-LDA-86), with the General objective of the Project is to secure the enhancement of national capabilities for pandemic influenza vaccine and to create the framework for pandemic vaccine distribution in the neighboring countries by registering according to the EMEA requirements, lead by Associate Professor Dorel Lucian Radu. The project also had a whole WP dedicated to pre-clinical immunogenicity and safety studies where the main objectives were to precisely define the specific standard operating protocols in preclinical models according to EMEA requirements. Preclinical immunogenicity studies aimed to offer a global view of host specific immune response to vaccination with two route of administration: i.m. and i.d. and the possibilities to evaluate the most efficient formulation and routes of the candidate vaccine. Preclinical safety studies aimed to establish the adequate protocols to identify and characterize potential toxic effects of a vaccine in order to conclude that it is reasonably safe to proceed to clinical investigation. The study design was to investigate the single dose toxicity in order to define maximum tolerated doses. The WP was leaded by CSII Dr. Crina Stavaru. She has also developed methodology during the projects "**The use of experimental medicine to study immune response and the identification of new therapeutic targets"** (PN 06150202 and "**Development of the methodology for preclinical testing of biological products"** (PN 09220201) .

It has to be noticed that two international projects **FASTVAC: A generic framework forum FAST production and evaluation of emergency Vaccines (**DG SANCO / FASTVAC - CSI Dr. Dorel Lucian Radu) and **Development and Sustainable Manufacturing of Adjuvanted Pandemic Influenza Vaccines in Developing Countries** (U.S. ASPR / BARDA / AMS - CSI Dr. Adrian Onu) have consistent sections of research on experimental animals.

The quality of research staff is also largely recognised. DR. Cristin Coman is the president of the the "**Romanian Association for Laboratory Animal Science**".

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83. **Iancu AD,** Radu BM, Radu M, **Stavaru C, Radu DL,** Double transgenic mice with type I diabetes - a model of diabetic neuropathy, *Joint Meeting of Immunology and Clinical Allergology*, 2010, Romania, Sibiu

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94. WHO influenza vaccine production capacity building grant Grant Agreement: SPHQ-LOA-86 / 24 June 2009 "Cantacuzino" National Institute for Research and Development in Microbiology and Immunology Bucharest – ROMANIA, Cancun, Mexic, Mai **2011**.

The continuous education of young researcher is the characteristic of the scientific policy of the team.

- Crina Stavaru, Orhan Rasid, Adina Daniela Good Laboratory Practice Training Course – curs de formare, Bucuresti, 17-23 noiembrie 2010:
- Grosu Melania – Health Protection Agency Porton Down, Anglia; : 29.05.07-5.06.07
- Iancu Adina Daniela - National & Kapodistrian University of Athens, Faculty of Science, School of Biology, perioada 9 iunie – 3 iulie 2008. Temele:
- BD FACSCantoII flowcytometer utilizand BD FACSDiva 6.1.1 software la BD Biosciences, 20-24 octombrie 2008, Heidelberg, Germania.
- Iancu Adina Daniela -Stagiu de pregatire "Advanced BD FACSCantoII and BD FACSDiva 6" 15-18 iunie 2009, Bucuresti.

REPRESENTATIVE PROJECT - PANDEMIC INFLUENZA VACCINE DEVELOPMENT

Emergency vaccine development is a complex range of activities covering different fields of biomedical R&D from microbiology and immunology to biotechnology and pharmacology. This activity benefits the most from the concept of integrating public health / research and development / production which was originally demonstrated in vaccination campaigns in 1913 and 1916. Thus, during the Balkan war, based on scientific observations, the ability to prepare a vaccine, and the concentration of decision in the hands of specialists, quarantine and vaccination of an entire army corps (50,000 men) was managed and the epidemic was stoped - from July 13, 1913 (first case of cholera) until August 5. This success had a major contribution to the establishment of the Cantacuzino Institute.

The same integrative approach was later used for the development of the pandemic influenza vaccine. In the years following the wave of avian influenza there was sustained focus on developing a vaccine for special circumstances manifested in national and international projects. The effort to obtain the Pandemic vaccine (2009) involved testing of several variants, a large number experiments and data analysis in a short time followed by decisions on next steps work which required the involvement of large numbers of people from all departments of the institute.

In 2005 during the wave of avian influenza, by mobilizing the collaboration of several laboratories from the Cantacuzino Institute and research fellows at the Center of Military Medical Science we succeeded in a very short time to characterize sequences of circulating viruses, isolated by colleagues at the Institute for Veterinary Diagnosis, and their possible origin, and thus the degree of risk could be assessed. Although a success, this underlined the necessity for pandemic preparedness by also producing a vaccine.

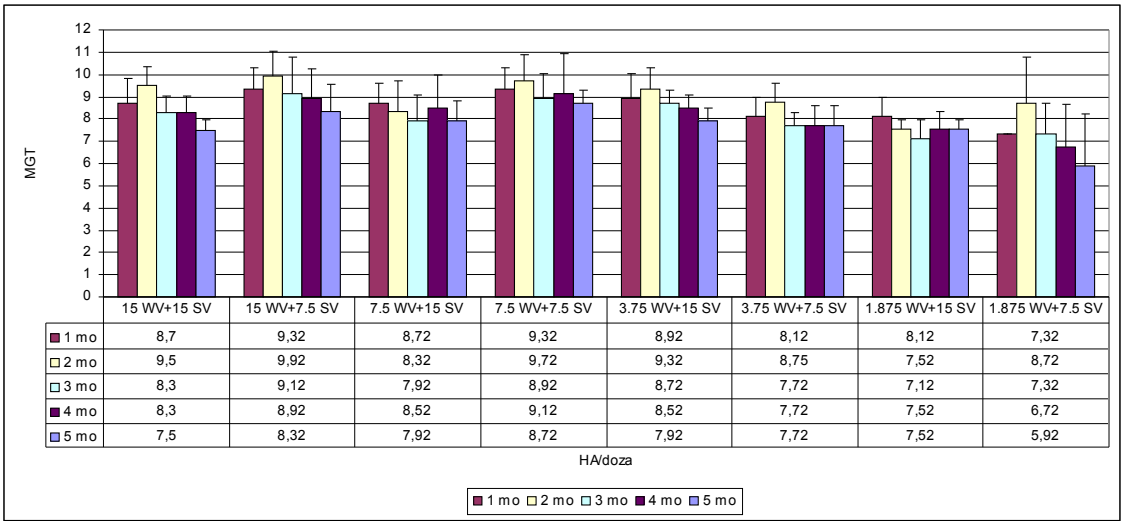
Therefore one of the priories of the institute was to improve development capacities in the field. In the absence of a strong specific industry in Romania, the development was focused on our own products. A modern vaccine requires dedicated activities and capacities for research and development. Beside the creation of a dedicated vaccine development infrastructure complete with pilot areas, modern equipment, and animal experiment facilities, development of laboratory technologies and validated operational procedures are also required. Also, establishing partnerships with other institutions with complementary activity is important from this point of view. Due to it's complexity, this meta-project was implemented as a result of several smaller project and grants. The formal activities started in 2006 with two projects, one national **"Experimental model of influenza virus strains of immunological research and clinical studies of human infection to establish a methodology for evaluation of influenza vaccines"** - Acronym: EVAGRIP CEEX 160 / 2006 (2006 – 2008) and one international "**Combating flu in a combined action between the industry and the public sector in order to secure adequate and fast intervention in Europe**" FluSecure, funded by DG-SANCO. The high amount of resources needed to build capacities were obtained from other external grants: **WHO influenza vaccine production capacity building grant** (Letter of Agreement SPHQ09-LDA-86), was dedicated not only for technology but also for the development of research

infrastructure. The grant was complemented by an **adjuvanted influenza vaccine development grant** with Infectious Disease Research Institute, Columbia Street, Suite 400 Seattle, WA 98103 (F16SUB-2010) subsidiary of the main grant 1 IDSEP100008-01-00 1124 funded by de ASPR/BARDA/AMS, „**Development and Sustainable Manufacturing of Adjuvanted Pandemic Influenza Vaccines in Developing Countries**".

The first studies were developed from the EVAGRIP project, and, as a result of the FluSecure project we could test the production pandemic vaccine candidates. Also, successful technological transfer of production and control methodologies of oil in water emulsions for adjuvanted influenza vaccine preparation resulted in generation of the framework for candidate vaccines which will be preclinically tested next year. One major achievement is the creation of a dedicated vaccine development infrastructure complete with pilot areas, modern equipment and animal experiment facilities. For instance in the In WHO influenza vaccine production capacity building grant all the phases of development of a modern product were present from the optimization of production technology and controls to non-clinical and clinical trials. The pilot area was built from WHO influenza vaccine production capacity building grant extended with the adjutant production equipment from IDRI/BARDA development grant. Also set-up of a GLP laboratory for animal experiments, currently in progress, was the result of national capacity building projects, WHO influenza vaccine production capacity building grant, and will benefit in its development from IDRI/BARDA development grant. This is improved with the benefits from the developments in the FastVAC project with technology transfer and an improved detergent spiting technology. These achievements also represented a major change in the development concepts, traditionally based on own resources, towards a modern collaborative integrated research. A more detailed description is presented below.

The project **"Experimental model of influenza virus strains of immunological research and clinical studies of human infection to establish a methodology for evaluation of influenza vaccines" - Acronym: EVAGRIP CEEX 160 / 2006** (2006 – 2008) **Coordinator:** National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" **Project Director:** Associate Professor Dorel Lucian Radu with the collaboration of Institute for Diagnosis and Animal Health - Project Coordinator: Dr. Aurelia Ionescu and the University of Agronomic Sciences and Veterinary Medicine Bucharest - Project Coordinator: Prof. Manuella Militaru. The project was aimed at the assessment of methodology for vaccines that could be applied in preventing influenza, comparative studies on routes of immunization by administration of influenza vaccine in humans, experimental influenza infection in animal models including transgenic mice and histology of harvested organs. Based on these, a study of influenza infection was published entitled **"Experimental study and clinical influenza infection and immunization with influenza vaccines"** - ISBN 978-973-708-332-6, *University Publishing House "Carol Davila", Bucharest , 2008.*

There were several outcomes from this project in different vaccine research fields. Regarding pandemic vaccine development, the main one was the opportunity to work with candidate pandemic vaccine strains (H5N1) and to verify and develop the requested techniques for immune response evaluation. A typical result is shown in the figure 1. A very important achievement was the establishment of a partnership with institutions having high competence in animal histopathlogy which was very important for the development of the project.



	15 WV+15 SV	15 WV+7.5 SV	7.5 WV+15 SV	7.5 WV+7.5 SV	3.75 WV+15 SV	3.75 WV+7.5 SV	1.875 WV+15 SV	1.875 WV+7.5 SV
1 mo	8,7	9,32	8,72	9,32	8,92	8,12	8,12	7,32
2 mo	9,5	9,92	8,32	9,72	9,32	8,75	7,52	8,72
3 mo	8,3	9,12	7,92	8,92	8,72	7,72	7,12	7,32
4 mo	8,3	8,92	8,52	9,12	8,52	7,72	7,52	6,72
5 mo	7,5	8,32	7,92	8,72	7,92	7,72	7,52	5,92

Figure 1: Immune response after i.m. administration NIBRG – 14 (first dose whole virus and second dose split virus)

The EU finaced FLUSECURE project "**Combating FLU in a combined action between industry and the public sector in order to SECURE adequate and fast interventions in Europe**" started from the observation that the main problem is that no vaccine can be produced in advance, since the exact nature of the virus is not known until the outbreak starts. Of all serious health threats, pandemic influenza is now felt to be the major one, especially since effective preparedness planning is lacking. As this is a cross-border problem, it needs a cross-border solution. This requires that in Europe the industry and public health authorities collaborate in jointly gaining control over the infectious disease. As an overall objective this project aims to enable manufacturing of the most effective pandemic vaccine in the shortest possible time in sufficient quantity for the EU population, by setting up a European network of public health institutes as the public sector input for a public-private partnership. Therefore this was developed as a consortia of EU institutes leade by Netherlands Vaccine Institute (Netherlands) which are: Health Protection Agency HPA (UK), Statens Serum Institute SSI (Denmark), National Institute for Health and Welfare (Finland), Norwegian Institute of Public Health (Norway), Cantacuzino Institute (Romania), National Centre for Epidemiology (Hungary), Slovenia National Institute of Public Health (Slovenia), Robert Koch Institute (Germany), Institut Pasteur (France). The duration of the project was 54 months: from February 2006 till July 2010.

Cantacuzino Institute contributed actively in two work packages 4 and 6. The work package 4 had the task of "**RAPID DEVELOPMENT AND PRODUCTION".** It consisted in construction of a library of likely H and N antigen types on a PR-8 backbone (or other helper viruses) either by reverse genetics or by conventional methods to make re-assortants with potential pandemic H and N antigen types (e.g., H5N1, H9N3, H7N7 etc.) . The H and N genes were cloned from wild-type viruses and produced as PR8 re-assortant viruses either using conventional or reverse genetic techniques. All the re-assortants were tested for attenuation in animal (ferret) models and the next step was the production additional reagents for each PR8 re-assortant and banking. PR8 re-assortants and reagents were released as a package to vaccine manufacturers. Cantacuzino Institute produced purified material from the following strains.

Subtype	*Strain*	*Vaccine Reference strain*	*Date of Ag production*
H9N2	A/Hong Kong/1073/99	wt	10/2007
H7N3	A/mallard/Netherlands/12/2000	NIBRG-60	11/2007
H9N2	A/chicken/Hong Kong/G9/97	NIBRG-91	09/2008
H5N1	A/Cambodia/R0405050/2007	NIBRG-88	09/2008
H2N3	A/mallard/England/727/2006	NIBRG-107	01/2009
H7N2	A/New York/107/2003	NIBRG-109	02/2009

The reagents were made in a collaboration between HPA NIBSC and CI, initiated early in the FLUSECURE project, which has been highly successful and has enabled FLUSECURE to produce a unique library of not only candidate vaccine viruses but also matched antigen standards (and antisera). Both parties are keen to continue the collaboration should funds become available. In the absence of secure funding, HPA NIBSC have issued a formal Letter of Commitment to CI expressing their intention to collaborate in the future should the means be available. The library was promoted in different meetings (Influenza pandemic reagent library. Presented at: Medical, Scientific and Historical Lessons from the Great Avian (H1N1) "Spanish" Influenza Pandemic of 1918: the 90th Anniversary. November 10, 2008. London, UK, O. G. Engelhardt, D. Major, K. Guilfoyle, E. Lupulescu, R. E. Johnson, J. S. Robertson, J. Wood, and the FLUSECURE consortium)

A interesting outcome of the project was the bulk antigen production for the pendemic virus derived reasortants soon after the pandemy was declared (H1swN1). At the request of HPA NIBSC, CI made bulk antigen against one classical and one non-classical H1N1 candidate vaccine virus (see Table 2). The X-179A antigen preparation has been filled for use as a reference standard for serology testing and is available from HPA NIBSC being in the benefit of the whole community for pandemic vaccine development. For the Cantacuzino Institute itself that material was used immediately in preclinical trials leading to the development of H1N1 vaccine in a timely fashion.

Subtype	*Strain*	*Vaccine Reference strain*	*Date of Ag production*
H1swN1	A/California/7/2009	X-179 A	07/2009
H1swN1	A/California/7/2009	NIBRG-121	07/2

A less major outcome from the project was the technology of predicting virus production yield in eggs using PCR. Prediction of the yield of a new candidate vaccine virus under production conditions in eggs usually requires time consuming experiments in embryonated hens eggs. CI has investigated a more rapid approach in which virus is grown in cell culture and yield assessed by measuring gene levels of HA and M protein using quantitative real-time PCR (qRT-PCR). Details of the work undertaken are given below, but, in summary, the assay enables intracellular production of virus to be compared to extracellular release (release of virus is necessary for vaccine production) and preliminary assessment of the ability of viruses to grow in chicken or mammalian systems. The test however requires careful design, with internal controls, use of several replicates and analysis of samples in a range of dilutions. Automation of the assay would help in this respect.

Interestingly by assessment of suitability for growth of 3 H1N1 (pdm) candidate vaccine viruses in eggs NYMC X-179A, a classical reassortant that is a moderate to good producer in eggs, NIBRG-121, a reverse genetics reassortant which is a poor producer, NIBRG-121XP, derived from NIBRG-121 by serial adaptation passage in eggs and which is a very good producer, the data correlated with those obtained in production. The NYMC X-179 was already in production for the pandemic vaccine when these data were obtained.

An assay which was derived from this one was was very fast and sensitive for assessing viral inactivation and was used in RIVERS project (Maria Elena Mihai, Cristina Tecu, Alina Elena Ivanciuc, Gheorghe Necula, Emilia Lupulescu, Adrian Onu. Survival of H5N1 Influenza virus in water and its inactivation by chemical methods. Archives of Microbiology and Immunology, vol, 70, nr.2, **2011**, pg 34-40).

The work package 5, "**EFFECTIVE VACCINES"** had the task to augment the immunogenicity of the current influenza vaccine using an adjuvant system and to establish an optimal immunization strategy giving rise to long-lived B- and T-cell responses using a simple vaccination regime. Different adjuvant systems were tested during this work. A typical result is shown in figure 2.

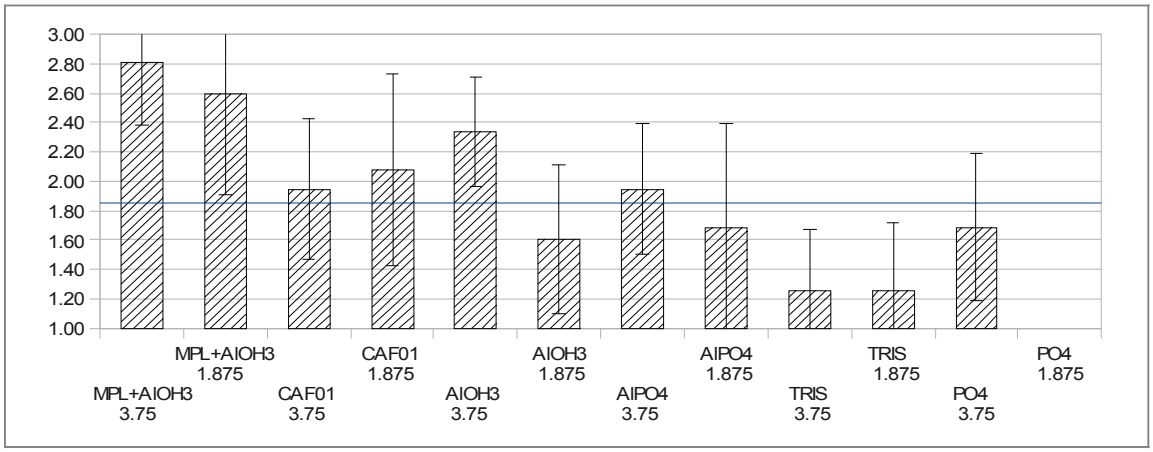


Figure 2: Fold increase after the first immunization (NIBRG 14) - MPL - monophosphoryl lipid, AlOH3 aluminum hydroxide adjuvant, AlOH3 aluminum phosphate adjuvant.

A major outcome from these tests was the development and validation of a microneutralization assay for for the evaluation of immune response which later allowed it to be used for nonclinical and clinical tests. Also participation is such a consortium increased our credibility, opening opportunities for other funds.

The best example was WHO influenza vaccine production capacity building grant 2009-2011 (Letter of Agreement SPHQ09-LDA-86), with the General objective of the Project is to secure the enhancement of national capabilities for pandemic influenza vaccine and to create the framework for pandemic vaccine distribution in the neighboring countries by registering according to the EMEA requirements, lead by Associate Professor Dorel Lucian Radu.

The project had three main objectives related to issues of pandemic vaccine development. Four categories of activities were performed in parallel since some of the development phases were already done or in progress four work packages were defined. In work package 1: Optimization of influenza vaccine production the objectives was to seek effective strategies for increasing the production capacity. In work package 2: Pre-clinical immunogenicity and safety studies the main objective was to define precisely the specific standard operating protocols in preclinical models according to EMEA requirements. Preclinical immunogenicity studies aimed to offer a global view of host specific immune response to vaccination with two routes of administration: i.m. and i.d. and the possibilities to evaluate the most efficient formulation and routes of the candidate vaccine. Preclinical safety studies aimed to establish the adequate protocols to identify and characterize potential toxic effects of a vaccine in order to conclude that it is reasonably safe to proceed to clinical investigation. The study was designed to investigate the single dose toxicity in order to define maximum tolerated doses. Autopsy and histopathological analyses of immune organs (spleen, thymus, lymphatic nodes) and pivotal organs (brain, heart, lung, and kidney) was supplied by contract, collaboration standard during EVAGRIP. Work package 3: Phase I clinical trials, was designed to assess the safety, tolerability and pharmacodynamics of the pre-pandemic influenza vaccine produced by Cantacuzino Institute. The trial was conducted in an inpatient clinic, where the subject cold be observed by full-time staff. The last work package, 4: Project management and technical support had the objective, developing pilot unit.

The grant stated in June 2009 with the nonclinical package on H5N1 derived reassortant but just two weeks later the H1swN1 pandemic was announced. The nonclinical results on H5N1 were continued, serving as preliminary results for building the vaccine dossier until the H1swN1. Fortunately, the involvement in FluSecure provided enough material in July to start a new set of preclinical trials to choose the best formulation and to obtain the vaccine in a timely fashion. Without the framework built as the result of this meta-project this would not have been possible. The WHO grand suffered several modification due to H1swN1 pandemics and some delays, but in the end a pilot area was also built allowing resulting in an increased capacity for R&D in vaccinology. Also this grant was complemented by the adjuvanted influenza vaccine development grant with Infectious Disease Research Institute, Columbia Street, Suite 400 Seattle, WA 98103 (F16SUB-2010) subsidiary of the main grant 1 IDSEP100008-01-00 1124 funded by de ASPR/BARDA/AMS, „Development and Sustainable Manufacturing of Adjuvanted Pandemic Influenza Vaccines in Developing Countries”. The new adjuvant will reduce the necessary biological material at least to the level identified in the

FluSecure project. After completion, Cantacuzino Institute will not only succeed to develop a modern emergency vaccine but will also end-up with a modern platform for the vaccine development.

In terms of capacity building, experimental laboratory animal work benefited from - EVAGRIP – project. A larger platform located in Baneasa is the result of a project to develop research infrastructure ("Upgrading of research infrastructure for growth, maintenance and use for scientific experiments and other laboratory animals Baneasa location, National Institute for Research Development in Microbiology and Immunology - BIOSCANT – Capacities Program - Modules I and II competition in 2008). This will also be the location of the upcoming GLP preclinical animal studies unit.

This approach consolidated Cantacuzino Institute's capabilities in vaccine development by bringing together complementary teams lead by specialists in influenza (such as Dr. Emilia Lupulescu and Dr. Viorel Alexandescu), manufacturing (such as Dr. Margareta Mazilu, Dana Bonea/Laurentiu Bebecila), quality control (Dr. Denisa Mihai), immunology (such as Dr. Dorel Lucian Radu, Dr. Crina Savaru, Dr. Aurora Salageanu), animal work (Dr. Coman Cristin) and analytical protein technologies (Dr. Adrian Onu).

These achievements have proven that based on institutional expertise and external partnerships the Cantacuzino Institute can be redefined as an international level actor in vaccine research.