EIN-L-001 Antimicrobial peptides: A promising alternative for anti-infective therapeutics

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Antimicrobial peptides (AMPs) can be found in almost all superior live forms and although they present a weaker antimicrobial activity than conventional or classic antibiotics and can be toxic by systemic ways, they carry the decisive advantage of acting by molecular mechanisms related to more universal and frequently no monogenic structures and functions for which it is difficult to develop a substantial microbial resistance. Conventional or classic antibiotics are generally chemical compounds isolated from microorganisms acting competitively to other microorganisms. They have been used for decades for combating infections in animals and humans. They present important disadvantages as toxicity at antimicrobial effective doses, induce the development of multi-resistant strains and finally they are poorly understood by the host immune system. Marine invertebrates lack acquired immune response and their principal humoral effectors against infection are antimicrobial peptides mainly for those lacking chitin structures capable to mechanically isolate infection nucleus. The antigenic challenge of marine invertebrates is enormous and it is expected that the peptidic arsenal available for combating infectious agents at the coastal environment be diverse and also that such antimicrobial actions can be effective (as direct killers or immunomodulators) against microorganisms infecting humans and animals. Cm-p5, a peptide derived from the marine mollusk Cenchritis muricatus shows a significant fungistatic activity against pathogenic Candida albicans while exhibiting low toxic effects against a cultured mammalian cell line. Cm-p5 as characterized by circular dichroism and nuclear magnetic resonance (liquid) revealed an alpha-helical structure in membrane-mimetic conditions and a tendency to random coil folding in aqueous solutions. Additional studies modeling Cm-p5 binding to a phosphatidylerine bilayer in silico and isothermal titration calorimetry using lipid monophases demonstrated that Cm-p5 has a high affinity or the phospholipids of fungal membranes, only moderate interactions with a mammalian membrane phospholipid, low interaction with ergosterol and no interaction with chitin. Adhesion of Cm-p5 to living C. albicans cells was confirmed by fluorescence microscopy with FITC-labeled peptide.

Key words: Antimicrobial peptides, anti-infective therapeutics
The nucleolus is an organelle involved in ribosome biogenesis and other recently found functions as aging and cell cycle control. The absence of a nucleolus in the parasite Giardia lamblia prompted us to study its presence, since it was considered the only eukaryote without nucleolus. Using several techniques and markers, it was demonstrated a nuclear peripheral, fibro-granular ribonucleoprotein, containing fibrillarin, rRNA while lacking DNA material in both nuclei of trophozoites of G. lamblia. It turned out to be a very small, and probably the smallest, nucleolus present in each nuclei in the parasite. This finding has been later confirmed. However, the presence of nucleoli in other basal eukaryotes remained unknown. Therefore, using similar approaches, nucleoli were then described at ultrastructural level in epimastigotes of Trypanosoma cruzi and Entamoeba histolytica trophozoites. As in G. lamblia, a ribonucleoprotein and fibrogranular material was found within the nucleus of both species. Here we present data on the nucleologenes of these parasites, by using light and electron microscopy silver staining for nucleolar organizar. Results indicate that nucleolus does not disperses through cell division. Moreover, other free-living unicellular protists as Chaos, also show nucleolar material within the nucleus. (UNAM-DGAPA-PAPIIT IN220713).

**Key words:** entamoeba histolytica, giardia lamblia, nucleologenesis, nucleolus, trypanosoma cruzi
The broad spectrum of symbiosis includes parasitism: an association of two organisms, one of which (the parasite) lives in, or on, another organism (the host) - to the detriment of the host. Our studies to elucidate the ultrastructure of filariae - parasitic nematodes of man and animals - have revealed that these nematodes are themselves hosts of bacterial endosymbionts (Wolbachia). These Gram-negative bacteria are transovarially transmitted during the life cycle of the filarial, show tropism for the hypodermal tissue in all stages in the life cycle of filariae and for the germinal tissue of the females and appear to be essential for normal oogenesis and embryogenesis. Since Wolbachia have also been implicated in the pathogenesis of filarial infections, the host-parasite paradigm required modification to include this third component. Subsequent studies have shown that, in addition to Wolbachia, the filaria Onchocerca volvulus also harbors virus-like particles in the metabolic portion of its muscle cells, thus adding the fourth element to the paradigm. 

These findings have shown that some nematodes are susceptible to invasion by several types of prokaryotic organisms, rendering the host-parasite relationship that exists during filarial infections far more complicated than has hitherto realized. Furthermore, they indicate the need to modify the classical paradigm of filarial infections to include the consideration of the following series of interactions: vertebrate host-filaria; filaria-Wolbachia; vertebrate host-Wolbachia; the virus-like agent- filaria; virus-like agent-vertebrate host; and virus-like agent-Wolbachi. The nature of these associations requires further elucidation, especially the contribution that the organisms harbored by filariae contribute to the pathological manifestations of infection in the vertebrate host.

**Key words:** filariasis, wolbachia, intracellular endosymbionts, virus-like particles, host-parasite interactions
Serotype prevalence of group B streptococci (GBS) in a cohort of pregnant women with aerobic vaginitis in South Africa

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Introduction: The composition of the human vaginal microbiota may be influenced by hygiene practices, age, infections, sexual activity and hormonal changes e.g. pregnancy. Aerobic vaginitis (AV) refers to a disruption of the normal vaginal microbiota accompanied by an increase in vaginal pH and inflammation. Aerobic bacteria commonly associated with AV include GBS, known to be associated with adverse pregnancy outcomes and neonatal morbidity. GBS serotypes have been reported to differ in pathogenicity amongst women with different geographical location. The objective of this study was to establish the serotype prevalence in a cohort of pregnant South African women. Materials and methods: Ethics clearance for the study was obtained and 301 mothers gave informed consent to participate in the study. High vaginal swabs were collected and GBS isolation confirmed by standard procedures. Serotyping was achieved by the use of Streptococcus Group B typing sera. Results: Six different serotypes were identified, with serotype V predominating (66.7%), followed by serotype III (21.1%). One isolate from each of serotypes Ia, II, IV and IX were found. Serotype V showed antimicrobial resistance to several antibiotics used in prophylaxis. Conclusion: The colonisation of resistant GBS serotypes during pregnancy could pose a risk for maternal and neonatal morbidity in this population.

Key words: Serotype prevalence, streptococci (GBS), pregnant women, aerobic vaginitis, South Africa
Kidney infections caused by mycobacteria are chronic evolution, these could compromise both kidneys with subsequent renal failure. The main causal agent of these infections is Mycobacterium tuberculosis, however there are other non-tuberculous mycobacterial species (MNT) that may cause similar clinical symptomatology. The aim of our work is to point out the diagnostic significance of urinary tract infections caused by the genus Mycobacterium, since in many cases to not think about this type of infection these infections can progress to cause diseases spread and develop complicated.

Key words: Urinary tract infections, mycobacterium
Introducción: Con posterioridad a la epidemia del 2006 en Cuba se produjeron pequeños brotes de Dengue en algunas provincias del país, entre los que se encuentra el ocurrido en la provincia de Camagüey en el 2010. Teniendo en cuenta que en este a diferencia de otros brotes o epidemias no observamos casos severos nos trazamos como objetivo, la identificación de los serotipos causantes del brote, la caracterización o determinación de las secuencias de infección involucradas; así como la determinación de la duración de los anticuerpos IgM y el estudio de la presencia de signos y síntomas clínicos en la convalecencia tardía de la enfermedad. Materiales y Métodos: Para cumplimentar los objetivos se empleó la Inmunofluorescencia indirecta, PCR, Elisa de detección de anticuerpos IgM e IgG, además de la técnica de Neutralización por Reducción del Número de Placas. Resultados y Discusión: Los resultados arrojaron que en este brote circularon los serotipos 3 y 4, siendo mayor la circulación del serotipo 3. Las secuencias virales de infección DEN-2/DEN-3, DEN-1/DEN-2/DEN-3 y DEN-1/DEN- 2/DEN-3/DEN-4 se relacionaron con el cuadro leve de la enfermedad. Se observó la duración de los anticuerpos IgM entre 2-6 meses del inicio de los síntomas. Los dolores articulares, el decaimiento y la cefalea fueron los síntomas post-dengue más observados en los individuos con infecciones secundarias. Conclusiones: Estos resultados incrementan el conocimiento de la enfermedad y puntualizan el rol de los anticuerpos heterotípicos cros reactivos en la severidad de la enfermedad.

Key words: Virological and serological characterization, dengue, Camagüey
EIN-O-004  PHB-01 purification using a combination of methodologies liquid-liquid extraction and affinity chromatography

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HB-01 plantibody (PHB-01) is a murine antibody expressed in a non-commercial cultivar tobacco plants. It is routinely used as immune-reagent in the production process of Cuban vaccine against Hepatitis B virus. However, PHB-01 large scale purification from plant biomass imposes several difficulties, especially those related to the employment of a liquid-liquid extraction system, the low expression level in tobacco plants and the chromatographic matrixes cost because of its short timelife. In order to solve these issues a novelty two-aqueous phase systems for protein extraction were studied. The vegetal extract obtained from the best performance system (10% PEG 4000/15% potassium phosphate, at pH 5,5) was chosen for a purification study with different affinity matrixes. This extract was applied in three chromatographic matrixes: Prosep vA/Ultra, High-Flow Protein A Sepharose and CIM r-Protein A-1 monolithic column. Recoveries of 89,3 ± 9,3 % and 58,6 ± 4,7 % were obtained with the first two matrixes respectively. On the third matrix recoveries of 53,8 ± 18,4 % and 17,1 ± 26 % were achieved when the applied samples were 10 and 260 mL of extract, respectively. More than 90% of purity was obtained in all eluted samples. Finally, taking account these experiments, it can be said that the two-aqueous phase system (10% PEG 4000/15% potassium phosphate, at pH 5,5) with Prosep vA/Ultra or High-Flow Protein A Sepharose can be efficiently employed for PHB-01 obtainment.

Key words: PHB-01 purification, liquid-liquid extraction, affinity chromatography
Cloning and expression of gene l1 of the Human papilloma virus type 16

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Human papillomavirus type 16 (HPV16) is a virus that infects anogenital mucosal epithelia. In some cases, in women, infection can progress to cervical cancer. The virus genome can be divided into an early and late coding region. The late coding region contains the l1 and l2 genes. The major capsid protein L1 contains the immunodominant neutralization epitopes and can autoassembles to form virus-like particles (VLPs) in bacterial cells. Therefore, HPV L1 capsid proteins have been well investigated as potential vaccine candidates. We amplified the l1 gene with and without stop codon by PCR from a genomic DNA sample of a cuban diseased patient with HPV16. The amplification product was cloned into the Ncol - BgIII sites of the pBAD / Myc-HisA expression vector, and into the Ncol - HindIII sites of the pET28a expression vector. The l1 gene without the stop codon was cloned in frame with the coding sequence for the myc-6xHis epitopes in the pBAD / Myc-HisA vector. This was extracted by digestion with the Ncol-ScaI enzymes and subcloned into the Ncol- blunted HindIII site of pET28a. L1 protein production was determined by coomassie stained SDS-PAGE and by western blot in E.coli Rosseta, in E. coli BL21 (DE3) was only possible by western blot, whereas in E. coli Top 10 was not detected. The presence of rare codons in the l1 gene is a limiting factor for expression in E. coli, therefore a synthetic gene would be an alternative that would allow higher levels of production of HPV16 L1 protein.

Key words: Cloning, expression of gene l1, Human papilloma virus type 16
Human papillomavirus type 18 is closely associated with the development of human cervical carcinoma, which is one of the most common causes of cancer death in women worldwide. At present, HPV genotype 18 accounts for about 14.6% of all HPV infections and is the second most prevalent type, according to the population studied in Havana, Cuba. The commercial vaccines against HPV infection are based on the L1 major capsid protein. In this work, we expressed HPV18 L1 in Escherichia coli, which is an efficient and inexpensive platform used to produce high amounts of recombinant proteins. The gene encoding the major capsid protein L1 of the high-risk HPV type 18 was isolated by PCR as a 1521 bp fragment from a Cuban female patient and inserted into pET3a and pLEX vectors to obtain the recombinant expression vectors pETHPV18-L1 and pLEXHPV18-L1, respectively. pLEXHPV18-L1 was introduced into E. coli GI724 and E. coli BL21(DE3). The expression of HPV18 L1 was not detectable when tryptophan and IPTG was added to E. coli GI724 and BL21(DE3), respectively. However, HPV18 L1 was expressed when E. coli Rosetta(DE3) and Origami (DE3) were used as host, taking into account that the L1 gene has a high frequency of minor codons in E. coli and 14 cysteine codons, respectively. HPV-18 L1 was also expressed as a fusion protein with a completely removable N-terminal His6 affinity tag. This work provides necessary basis for preparing HPV-18 L1 vaccine to prevent persistent HPV18 infections and cervical cancer in Cuba.

**Key words:** human papillomavirus, L1 major capsid protein, hpv type 18
Thorough characterization of microorganisms used to produce vaccines is essential to assure the quality of the final product. Regulatory authorities demand to demonstrate the authenticity of strains used, especially if they are intended for human use. This is usually achieved based on certain stable phenotypic or genotypic characteristics which will vary for each group of microorganisms. Current standard techniques for identifying Leptospira spp. are based on antigen-antibody reactions; they are either expensive or cumbersome. Pulsed Field Gel Electrophoresis in combination with Analysis of Restriction Fragment Length Polymorphisms have been used to identify Leptospira spp. to the serovar level. We have obtained characteristic DNA band patterns for each strain used to produce the active pharmaceutical ingredient of vac-SPIRAL® vaccine at Finlay Institute. Microorganisms were cultured in EMJH medium for 7 days at 28 °C and 130 rpm. Samples were prepared using Backit® ready to use kit, following manufacturer's instructions and digested with 10 units of Not-I endonuclease. Electrophoresis was performed in a miniaturized Transversal Alternating Gel Electrophoresis chamber at 20 °C and 10 V/cm, using switching times from 3 to 22.8 s during 8.4 hours. Patterns containing 12 to 15 bands ranging from 20.5 to 1135 kb were obtained allowing to differentiate one strain from each other. The method proposed here is faster than previously reported for obtaining Not-I patterns of Leptospira spp. due to the use of miniaturized chamber and could be a useful tool to test the authenticity of Leptospira spp. strains.

**Key words:** leptospira interrogans, transversal alternating field electrophoresis, vaccine strains authenticity
EIN-O-010  Development of an anti-IL-15 vaccine

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The IL-15 is an immunostimulatory cytokine that is expressed on uncontrolled form in several autoimmune and inflammatory diseases as Rheumatoid arthritis and psoriasis. Inhibition of IL-15-induced signaling could be clinically beneficial. In our approach to inhibit IL-15 we tested active immunization with structurally modified IL-15 in Aluminum, Montanide and Freund adjuvants in non-human primates and we obtained high titers of neutralizing antibodies against native human IL-15. Recombinant human IL-15 was obtained from E. Coli. After purification steps, the protein was obtaining with more than 95 purity percent and adjuvated with Aluminium hydroxide, Montanide and Freund Monkeys were immunized by subcutaneous injections of adjuvanted-recombinant IL-15 or control preparation. Sera were collected 15 days or 3 month after the third immunization. The antibodies titer was determinate by ELISA and CTLL-2 cell proliferation assays. Native human IL-15 was obtained from R&D and the recombinant monkey IL-15 was obtained from E. Co Active immunization with adjuvated recombinant modified IL-15 induces high titers of IgG neutralizing antibodies against human IL-15. Sera from immunized animals inhibit IL-15-dependent cell lines proliferation assays, suggesting that active immunization is capable of breaking immunological B cell tolerance. Aluminium and Montanide showed the similar levels of antibodies, but Aluminium induce the better neutralizing response. The immune response is regulate and decrease to baseline 2 months after immunization but the booster recovery high titer of antibodies. Furthermore, we demonstrated that sera generated by immunization with recombinant human IL-15 recognize and neutralize the IL-15 of monkey. No chance in hematological and biochemical parameters was observed in immunized animals. Conclusions. Vaccination with recombinant modified human IL-15 was able to induce neutralizing antibody response to native IL-15 in non-human primate

Key words: human il-15, non-human primates, antibodies, immunization
EIN-O-011 Integrating the patients' perspective in chronic disease care: Experience from Turkey

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The health agenda in Turkey is shifting from communicable and vaccine preventable diseases to non-communicable diseases (NDCDs). This is the first qualitative study in Turkey that examines the health system from the perspective of a patient with NCDs. We aimed to describe the clinical context in which patients and clinic staff together negotiate the management of diabetes/cardiovascular disease (DM/CVD). This study is part of a larger European Commission (EC)-funded project (MedCHAMPS, 2009-13). Data collection included observations in four clinics, plus interviews with 20 patients, 11 patient relatives and 8 health providers. We found that, in general, people were aware of the risk factors of their chronic diseases and aware of ways to protect against deterioration. When talking about the quality of health services, non-medical concerns stand out most clearly for patients and their relatives. Regarding patient-medical staff relations, the impression of a power based medical hierarchy was strongly felt by patients attending public facilities. Patients and providers had different perceptions regarding the adherence of patients. Two recurrent themes emerged from our analysis: one relates to the fact that seeking for a better service is constant and second “lack of trust” towards the quality of services as well as between patients and providers. The results of the current study indicate that at health service delivery level, the Turkish system is inadequate to address the health needs of the chronic disease patients and their relatives. Patient’s central role and responsibility in health care delivery should be encouraged and health care for chronic conditions must be re-oriented around the patient and family.

Key words: Integrating the patients, chronic disease care, Turkey
Streptococcus pneumonia (pneumococcus) remains a leading cause of bacterial infectious diseases mainly in children under five years of age. Due to the serotype replacement observed after the introduction of pneumococcal conjugate vaccines (PCV), the use of pneumococcal proteins as active carriers constitutes a promising alternative to expand PCV coverage. In this study recombinant fragments of PspA clades 1 and 3 were purified and conjugated to capsular polysaccharide serotype 6B. The PspA purification involved the following chromatographic sequence: Q-Sepharose (anion exchange), IMAC- Sepharose (immobilized metal-ion affinity chromatography) and SP-Sepharose (cation exchange). In order to conjugate the polysaccharide to the purified protein, the pneumococcal capsular polysaccharide serotype 6B was first fragmented through acid hydrolysis, and then activated through the periodic oxidation of vicinal hydroxyl groups followed by the introduction of an amino spacer (1, 8-diaminocan). The conjugation method used DMT-MM as activating agent of protein carboxyl groups. At the end of the purification steps, the purity of rPspA3 and rPspA1 reached a 95% although the recovery was low for both proteins, around 6%. The modifications of polysaccharide 6B prior to conjugation finished with the introduction of 4 molecules of amine per polysaccharide molecule, which further allowed efficient coupling to proteins rPspA1 and rPspA3. About 25% of polysaccharide was incorporated in the conjugate fraction. Conjugates to both proteins were characterized by size exclusion chromatography and SDS-PAGE. Overall our results support the use of PspA as carrier in a conjugate vaccine where both components, the polysaccharide and the protein, act as antigens.

**Key words:** streptococcus pneumonia, pneumococcal surface protein a, pneumococcal capsular
EIN-O-013 In vitro study of anti-tuberculosis activity of plant extracts of the Peruvian Amazon

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Introduction. The WHO (World Health Organization) declared to Tuberculosis as a global emergency since 1993. The Peruvian Amazon is one of the areas of the planet with greater diversity of plant species, of which less than 1% have been studied. Therefore, the Peruvian flora is, without doubt, an alternative source of anti-mycobacterial extracts and metabolites. The development of a line of research will be possible when concrete bases as the present study is established.

Material and method. The leaves of species Juglans neotropica Diels. Piper aduncum L., Croton lechleri Müll. Arg., Lantana camara L., Annona cherimola Mill, Annona muricata L. y Jatropha gossypifolia L., were collected according to the current chemotaxonomy. The chloroform and ethanol extracts were prepared. The bioactivity against Mycobacterium tuberculosis H37Rv of extracts were determined by antimycobacterial screening based reduction of alamar blue at three concentrations (10, 100 and 1000 µg/ml); following the original protocol Collins and Franzblau with some modifications. The minimum inhibitory concentration (MIC) in vitro of the extracts with bioactivity against Mycobacterium tuberculosis H37Rv in the concentration range of 2000-15.63 µg/ml was determined by assay in microplate Blue Alamar; following the original protocol Collins and Franzblau. Results. The antimycobacterial screening based on Alamar Blue determined that the 14 studied extracts (100%) showed bioactivity against Mycobacterium tuberculosis H37Rv to 1000 µg/ml. The microplate assay with blue Alamar determined that the ethanolic extract of Piper aduncum L showed a MIC = 31.5 µg/ml

Key words: In vitro study, anti-tuberculosis activity, plant extracts, Peruvian Amazon
Purification of p24 kDa protein of the human immunodeficiency virus type 1 from a new formulation of inactivated antigen

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24 kDa protein can be obtained by purifying HIV-1 natural antigens by immunoaffinity chromatography. The purification procedure has not changed; but changes have been made in the process of obtaining antigens. The aim of this work was to demonstrate that the new method for obtaining HIV-1 natural antigens did not affect the antigenic and immunogenic properties of the purified p24. HIV-1 antigen was purified by immunoaffinity chromatography. Protein purity was determined by electrophoresis followed by silver staining. Molecular weight marker and pattern of HIV-1 protein was used to estimate molecular weight of purified protein, by measuring the relative mobility. For immunogenic characterization of purified protein, rabbits were inoculated with the purified product and the obtained serum was analyzed by ELISA. Antigenicity is confirmed by Western blot analysis, which revealed immunologically with a positive serum of HIV-1. Silver staining showed bands at 55 kDa and 24 kDa. The molecular weight of purified protein was 24 kDa. Titration of rabbit serum by ELISA reached titles of 1: 1250. Western blot assay showed strong reactivity against two proteins corresponding to p55 and p24 of HIV-1. Antigenic and immunogenic properties of purified p24 were not affected.

Key words: hiv-1 p24 protein, purification, affinity chromatography, immunization
Phage carrying human domain antibody (Dab) fragment recognizes Gamma Interferon in the sera of tuberculosis patients

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Introduction: Human tuberculosis (TB), caused by infection with Mycobacterium tuberculosis, was declared a global emergency by the World Health Organization. One essential factor for controlling the spread of this disease is the ability to diagnose infection in its early stages. Interferon (IFN)-blood tests may improve the current level of diagnostic accuracy for tuberculosis infection. Phage display can be used for antibody library production and screening. Several phage display libraries are currently available, among them, human domain antibodies libraries which may recognize a wide repertoire of antigens. Materials and Methods: In this work, we obtained phages expressing fragments of human domain antibodies (Dab) against Gamma Interferon. For biopanning process, ELISA plates were coated with IFN (Sigma, USA) at concentrations of 10, 5 and 1 μg/μL respectively in each round. For the reactivity screening, Gamma interferon (SIGMA) was used. Twelve phages were selected by its positive recognition to IFN. Plasmid DNA of each clone was purified by alkaline lysis (QIAGEN, USA) and sequenced (MACROGEN SA, Korea). One clone (B10) was selected by its strong recognition to IFN determined by ELISA using different phage concentrations. Immunological assays were carried out with the sera from five tuberculosis patients (confirmed by sputum and X-Ray) and 3 healthy controls. Measurement of IFN in the sera was performed by ELISA, using anti-IFN monoclonal antibody (Sigma, USA) as capture antibody and phage B10 as detection antibody. Results: The fragment of monoclonal antibody identified shows high affinity and selectivity for the antigen Gamma Interferon. This antigen could be detected in the sera from tuberculosis patients. Conclusion: This technology offers an advantage over traditional monoclonal antibodies (mAbs) produced by relatively expensive and time consuming techniques. Rapid diagnostic test could be performed to evaluate the progression of tuberculosis disease.

Key words: human domain antibody (Dab), Gamma Interferon, tuberculosis patient
Genotypic diversity of extended spectrum Beta-lactamase (ESBL) producing Escherichia coli isolates in a clinical surgical hospital in Havana, Cuba

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Introduction: Escherichia coli is the species most frequently associated with clinical infections, the strains which produce Extended Spectrum Beta-lactamase (ESBL) are often multidrug resistant and have been responsible of the increase of morbidity and mortality in hospitals. The objective of this study was to determine the genotypic relation of ESBL-producing E. coli recovered in a Clinical Surgical Hospital in Havana, Cuba. Materials and Methods: A retrospective study was conducted which were analyzed 35 clinical isolates E. coli collected in 2012. Antibiotic susceptibility was determined by standard disk diffusion and was confirmed the presence of ESBL using double disk diffusion test (DDD), the presence of genes was investigated by Polymerase Chain Reaction (PCR) and genotypic relation by pulsed-field electrophoresis (PFGE). Results and Discussion: The ESBL was detected in 35 E. coli isolates, of these 14 strains carried the blaTEM genes, 4 blaSHV genes and 18 blaCTX-M genes. The genotypic relation in the Internal Medicine ward showed four genotypes of which two clones are genetically related (similarity 80%) in the urology ward were detected nine genotypes of a total of 12 isolates and the Intensive Care Unit ward were detected 8 genotypes of a total 10 isolates in these last two wards isolates were genetically unrelated. These results demonstrate the genotypic diversity among isolates of ESBL-producing E. coli. Conclusions: This is the first report showed genotypic diversity of ESBL-producing E. coli in Cuba, suggesting potential cross-transmission thus highlighting the need for implementing control strategies to prevent dissemination of these enzymes in this one hospital.

Key words: escherichia coli, extended spectrum beta - lactamase (esbl), genotypic relation
EIN-O-019  Broad and functional T cell immune response following immunization with hepatitis C virus proteins-based vaccine formulated with polyester beads

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Introduction: Hepatitis C virus (HCV) infection is a major worldwide problem. Chronic hepatitis C is recognized as one of the major causes of cirrhosis, hepatocellular carcinoma and liver failure. However, even if a new wave of directly acting antivirals promise to overcome the problems of low efficacy and adverse effects observed for the current standard of care, an effective vaccine would be the only means to definitively eradicate infection and to diminish the burden of HCV-related diseases at affordable costs. Bioengineered biodegradable bacterial polyester beads have the potential advantages as vaccine delivery system as their size, versatility, inherent biocompatibility with living tissues. The ability of the Core-beads to enhance specific mouse immunity against HCV antigens was demonstrated. Materials and Methods: Polyester Core-Beads were provided by Polybatics, New Zealand. Core-beads were produced in E. coli. An immunization schedule was carried out in BALB/c mice and Core-Beads were administered intramuscularly, mixed to E1, E2 and NS3 recombinant HCV proteins. Core-Beads formulations were administered three or five times every fifteen days. Antibody response was measured by ELISA and T cell response was tested by ELISPOT and challenge with a surrogate virus model. Results: Three doses of Polyester beads-Core formulated with E1, E2 and NS3 recombinant antigens was able to induce an IFN- secreting and functional T cell response able to control the viremia after challenge with recombinant vaccinia virus. Conclusion: These results suggest that biopolyester beads could be an efficient vaccine delivery system able to increase specific cellular immune response against HCV. Therefore, polyester beads system might contribute to the development of a successful vaccine against HCV.

Key words: hcv, biopolyester beads, vaccine delivery system, mice, cellular immune response
La Resistencia mecánica es una propiedad de la matriz de purificación que está directamente relacionado con los problemas de la velocidad de flujo. Por ejemplo, una alta velocidad de flujo puede provocar la deformación de la partícula por compactación incrementado la resistencia al flujo y probablemente el desprendimiento del ligando. Por lo tanto, las perlas de la matriz deben cumplir con los parámetros establecidos por el fabricante para prevenir la compactación durante el proceso de purificación. Por tal razón fueron analizadas y comparadas en términos de resistencia mecánica la Sefarosa CL-4B, Applipurosa CL-4B y diferentes lotes del inmunoadsorbente CB.Hep-1. Se realizó teniendo en cuenta los siguientes parámetros (P/L, 15 cm/h; h de la matriz, 10 cm; P expresado en cm H2O; LF, desde 80 hasta 150 cm/h y como solución tampón fue usada 0.5M de NaCl). Como principales resultados se obtuvo que el flujo lineal de compactación más frecuente en la Sefarosa CL-4B y en los inmunoadsorbentes fue 123.82 y 138.80 cm/h, respectivamente. Por otro lado, la capacidad de elución y el desprendimiento de ligando fue 498.5±129.3 ?g/mL y 1.7±1.0 ngIgG/gAg, respectivamente. Este trabajo demuestra, que la activación de la matriz y el proceso de inmovilización del AcM CB.Hep-1 no provocan daños mecánicos en la matriz y por consiguiente en los parámetros evaluados en la cromatografía de inmunoadsorbente.

**Key words:** mechanical resistance, Sepharose gels, HBsAg purification
EIN-P-001 Purification of the protective components of Bordetella pertussis, using Fractogel media

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A general procedure using Fractogel media for purifying biologically active the pertussis toxin (PT) and pertussis toxoid (PTd), from Bordetella pertussis culture supernatants was described. This procedure allowed separation of both PT and PTd proteins from the filamentous proteins hemagglutinin (FHA) and pertactin (PRN) by a rapid single-step cationic chromatography. In a second chromatographic step, PRN from the cationic flowthrough was concentrated and purified on an anionic column. SDS-PAGE and Western blot analyses confirmed the high purity (95%) and immunogenic reactivity from all the purified antigens. The chromatographically purified PT protein retained its immunogenicity as showed by the Chinese Hamster Ovary clustering (CHO) assay, with a clustering endpoint at 2.45 pg/well. Moreover, PTd did not promote clustering at the highest concentration (1.6 µg/well). These results demonstrated that the protocol described for purifying the PT/PTd proteins can be used without affecting their biological activity, after two years of storage at -20°C. In addition, other pertussis antigens like FHA and PRN immunogenically reactive can be obtained. This paper constitutes the first report using the highly selective Fractogel media for purifying pertussis antigens. This one-step chromatography method reduces the time, cost and labor needed for purification of both PT and PTd proteins, which also may be very useful in the development and production of an acellular pertussis vaccine containing these antigens as safe components.

Key words: bacterial toxin, bordetella pertussis, chromatography, protein purification, protein stability
Introducción: Vibrio cholerae, serogroups O1 and O139, is the causative agent of cholera diarrheal disease. Much of the research aimed to develop oral cholera vaccines is directed to the production of live attenuated strains, such as the V. cholerae O139 TLP01 and TLP05 strains. If these strains would be used as active pharmaceutical ingredients for vaccine development, it is expected that vaccinees will excrete these strains in the feces. Microorganism persistence fundamentally depends on its ability to adapt and develop survival strategies. This work was aimed to model the environmental performance of these two strains. Their ability to produce different types of biofilms, to acquire rugose phenotype and to resist different environmental stress conditions such as the presence of chlorine, detergents or high salt concentrations, were evaluated in vitro. Concluding remarks: Attenuated Vibrio cholerae O139 strains TLP01 and TLP05 had limitations in the mechanisms that regulate biofilm and EPS formation in vitro, osmotolerance and susceptibility to detergents. The impact of these properties under other experimental conditions near those of the natural ecosystems should be further studied, in spite of these mechanisms being expected to influence the vibrios environmental performance to certain extent. The results indicate that these two strains have properties which limit their behavior in vitro, and such properties could act as containment for the biological agent used as active pharmaceutical ingredient for a vaccine candidate.

**Key words:** In vitro study, environmental performance, vibrio cholerae O139
Excretion of the vaccine strain Vibrio cholerae 638 in the feces of Cuban pediatric subjects

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Entre las medidas de control para la prevención del cólera, destacan aquellas dirigidas a garantizar fuentes de agua potable y saneamiento, pero estos son verdaderos desafíos para los países en desarrollo. La vacunación exitosa es también considerada una manera de prevenir la enfermedad. En Cuba se ha desarrollado el candidato vacunal CV638 que se encuentra en fase de evaluación clínica avanzada, cuyo ingrediente activo es la cepa Vibrio cholerae 638. El CV638 fue evaluado en 2013 en sujetos de edad pediátrica con el objetivo de verificar su seguridad, reactogenicidad e inmunogenicidad, tras su administración a niños y adolescentes de entre 5 y 17 años. Dado que se trata de una vacuna que administra el agente biológico vivo atenuado por vía oral, también se estudió la proporción de individuos y la magnitud de la liberación del agente vacunal al entorno, producto de la excreción fecal, así como las características genotípicas del agente liberado luego de 3, 4 y 5 días de la vacunación. Se concluyó que el CV638 coloniza el tracto gastrointestinal pediátrico, se multiplica "in vivo" y se excreta vivo y atenuado en las heces del 79,5% de los sujetos receptores de la vacuna, proporción similar a la alcanzada en sujetos adultos. El agente biológico excretado es indistinguible del ingrediente activo de la vacuna, en cuanto a los diferentes caracteres estudiados. Igualmente, la concentración de vibiones por gramo de heces fecales en vacunados de edad pediátrica excretadores del agente vacunal alcanza títulos entre 0,01 y diez millones de UFC/gramo, lo que es indistinguible de lo previamente reportado para adultos.

Key words: vacuna de cólera, vibrio cholerae, cepas atenuadas, excreción fecal
El estudio de sistemas híbridos es uno de los retos de la química orgánica moderna, debido fundamentalmente a las numerosas propiedades y variadas aplicaciones que estos poseen. En este sentido la conjugación de esteroides a otras moléculas de relevancia química o biológica es un enfoque actual en la búsqueda de interesantes aplicaciones biomédicas, debido fundamentalmente a su amplia gama de actividad biológica e interacción específica con los receptores hormonales. En los últimos años ha sido posible la obtención de varios sistemas heterociclo-estroide con potenciales aplicaciones biológicas. Algunos de los ejemplos reportados corresponden a híbridos de esteroides con heterociclos fusionados por los anillos A y/o D del esqueleto esteroidal fundamentalmente, los cuales constituyen importantes intermediarios sintéticos, y han presentado potencial actividad biológica. El presente trabajo recoge la síntesis de nuevos compuestos del tipo -cetoéster-estroide, 3,4-dihidro-2 (1H)-piridona-estroide y 1,4-dihidropiridina-estroide. Con la obtención de los -cetoésteres correspondientes fue posible sintetizar los híbridos 3,4-dihidro-2(1H)-piridona-estroide; empleando diferentes modificaciones de la reacción de Hantzsch y métodos no convencionales como la síntesis asistida por microondas. El conjugado 1,4-dihidropiridina-estroide se obtuvo utilizando el reactivo de Vilsmeier-Haack. Todos los compuestos sintetizados fueron caracterizados mediante espectroscopía de Resonancia Magnética Nuclear. A partir de esteroides hidroxilados en las posiciones 11 y 17 del esqueleto esteroidal y empleando la reacción con la dioxinona fue posible obtener tres nuevos oxobutanoxi-esteroide. Se determinó que el uso de la radiación por microondas permitió reducir considerablemente el tiempo de reacción para sintetizar el nuevo híbrido 3,4-dihidro-2(1H)-piridona-estroide, aunque los rendimientos fueron similares a los obtenidos por el método tradicional. La reacción de Vilsmeier Haack demostró ser un método efectivo para obtener híbridos 1,4-DHP- esteroide. Con empleo de las técnicas de resonancia magnética nuclear fue posible corroborar la obtención de los compuestos sintetizados.

**Key words:** New methodologies, hybrid systems, heterocycle-steroid
EIN-P-005  Effect of extraction time in the purified pertactin from Bordetella pertussis

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El proceso de extracción de pertactina (Prn) tiene gran impacto en la producción de Prn para las vacunas acelulares contra la tosferina. Unos de los parámetros que influyen en este proceso es el tiempo de extracción de Prn de las células de Bordetella pertussis. En este estudio se evaluó el efecto del tiempo de extracción sobre la calidad de Prn purificada de Bordetella pertussis. Se evaluaron tres tiempos de extracción: 60, 90 y 120 minutos, manteniendo la temperatura a 60º C para cada condición. Se observó que a medida que aumenta el tiempo de extracción, la Prn es más susceptible a la degradación. Los resultados obtenidos del análisis estadístico utilizando la prueba de Student-Newman-Keuls mostraron que el tiempo de extracción influye en la concentración de Prn obtenida y los mejores resultados se obtuvieron con 90 minutos de extracción.

Key words: extraction time, Bordetella pertussis
Allergic diseases have a high prevalence worldwide, being the exposure to Blomia tropicalis one of their main causes in Cuba and America. Up to now, no adjuvanted vaccine has been developed against this mite. Allergen-specific immunotherapy is the only treatment to induce antigen-specific Treg cells and peripheral tolerance. In this work, two adjuvants: the new AIF/IP and the traditional Prolinem were used with Blomia tropicalis allergens, to develop four experimental vaccines (AIF/PI-extract, AIF / PI-rBlo t5, Prolinem-extract and Prolinem-rBlo t5). The vaccine formulations using AIF/PI were characterized by physico-chemical methods. Three doses of each vaccine were subcutaneously administered to female BALB/c mice followed by a challenge. Immunogenic and immunomodulatory capacities, protective effect and general toxicity of these vaccines were evaluated. By means of an indirect ELISA, IgG, IgG1, IgG2a and IgE allergen specific antibodies and cytokines were determined in serum and bronchoalveolar lavage, respectively. Daily clinical observations were also conducted. Both adjuvants boosted the immune response, being it higher in the variants that used rBlo t5. For both adjuvants, the IgE levels remained similar or decreased after challenge. AIF/IP and Prolinem adjuvants induced protection, not only systemically by the IgG1/IgE relation, but also locally by cytokines in bronchoalveolar fluid. It was accompanied by a moderate Th1 pattern. No vaccine caused toxic effects and only in the variants with Prolinem, some nodules were detected at the site of inoculation. These results demonstrate that the new adjuvant AIF/IP is capable to stimulate the immune response against Blomia tropicalis without discomfort.

Key words: allergic diseases, blomia tropicalis, vaccine, mite, adjuvants, immune response
EIN-P-007 Development of a PCR assay to detect cholera toxin genes in preparations of the living vaccine candidate attenuated CV638

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The live oral vaccine candidate against cholera CV638 is produced under Good Manufacturer Practices. The active ingredient of this vaccine candidate is the genetically modified strain Vibrio cholerae 638; developed by researchers at the National Center of Scientific Research, from the strain V. cholerae serogroup O1 biotype El Tor C7258 (Peru, 1991), by removing the cholera toxin genes (ctxAB). Since the strain 638 lacks these genes in its genome, the presence of ctxAB in vaccine preparations, would be given by a contamination with a toxigenic V. cholerae strain. The present study was aimed at designing a specific PCR to detect ctxAB genes in DNA isolated from preparations of the vaccine candidate CV638, artificially contaminated with toxigenic V. cholerae strain. The sensitivity of the optimized PCR assay was 1 pg of genomic DNA from toxigenic strain of V. cholerae C7258, corresponding to 200 genomic copies. The sensitivity of the PCR method for detecting toxigenic strains in CV638 vaccine preparations contaminated with a toxigenic strain was ~ 7 x 10^3 colony forming units of the toxigenic strain per dose of CV638. Once validated, this method could be used in the quality control of the production of the live vaccine candidate CV638.

Key words: live vaccine candidate v. cholerae 638, cholera toxin, quality control, pcr
Vibrio cholerae is an enteropathogenic bacterium, which causes cholera. Cholera toxin is a protein synthesized by V. cholerae and it is responsible for unchain the diarrheal process by its action on the intestinal cells. VC638 is the attenuated strain derived from a second generation of genetically modified strains derived from strain C7258 virulent V. cholerae O1 El Tor Ogawa; it is isolated in Peru, 1991. In the fermentation step of VC638 are many variables to be optimized. This paper makes a study of two important variables in the fermentation process, they are: culture medium and pH. It has been reported that tryptone peptone (TP) is the most culture medium used in the fermentation culture of VC638 strain (3), it was suggested to try a new variant of tryptone peptone culture (TPM) and assess growth to different pH conditions. Factorial design (22) was carried out with two experimental variables (pH, composition of the culture medium). The response variable was the specific growth rate. One Marubishi fermentator of 5L was used with temperature, agitation and aeration controlled. 16 fermentations, 4 were conducted in each culture condition. Specific growth rate for each variant was determined. This variable was compared using the F test (F = 0.9438, df = 84) and no statistically significant difference between them (p = 0.4233). The average TPM culture proved to be feasible for fermenting VC638 found. The slightly basic pH pH = 8 did not significantly influence microbial growth.

Key words: culture médium, pH variables, fermenter culture, vaccine, Vibrio cholerae VC638
El cólera es una enfermedad diarreica aguda causada por la presencia del Vibrio cholerae O1 en el intestino delgado por la ingestión de alimentos o aguas contaminadas. En particular, las vacunas atenuadas parecen ser una de las variantes más promisorias, para su prevención y aprobación, se hace necesaria su evaluación clínica. La agencia reguladora cubana [Centro para el Control Estatal de Medicamentos, Equipos y Dispositivos Medicamentos (CECMED)] tiene entre sus requerimientos la eliminación de la cepa vacunal en los voluntarios de los ensayos clínicos antes de ser dados de alta. Por tanto, es necesario determinar el patrón de susceptibilidad a los agentes antimicrobianos que se usan en la terapia de las cepas vacunales en cuestión. El objetivo de la presente investigación consistió en determinar la susceptibilidad antimicrobiana de las cepas vacunales de V. cholerae para lo cual se utilizaron los agentes antimicrobianos siguientes: azitromicina, tetraciclina y ciprofloxacino y se probaron tres métodos diferentes: el sistema semi-automatizado D.I.R.A.M.I.C-10, Concentración Mínima Inhibitoria y difusión en agar (Kirby Bauer). Se analizaron nueve cepas de V. cholerae, que incluyeron al candidato vacunal oral cubano CV638, así como otras tres cepas, obtenidas a partir de una cepa del serogrupo O139. Los resultados obtenidos por los tres métodos empleados, evidenciaron que las cepas analizadas son sensibles a los agentes antimicrobianos evaluados, los cuales pudieran ser utilizados para la descontaminación de la cepa vacunal en voluntarios que se presenten a los ensayos clínicos para su evaluación.

Key words: cólera, susceptibilidad, antimicrobianos, cepas vacunales
EIN-P-011  Stability test of two batches of VC638 at 5C and 25C as part of the process of obtaining technological development of a vaccine against Vibrio cholerae

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Vibrio cholerae es una bacteria enteropatógena, causante de cólera. La toxina colérica, proteína sintetizada por V. cholerae es la responsable de desencadenar el proceso diarreico por su acción sobre las células intestinales. La cepa atenuada VC638 procede de una segunda generación de cepas modificadas genéticamente derivada de la cepa virulenta C7258 V. cholerae O1 El Tor Ogawa, aislada en Perú en 1991. El presente trabajo realiza un estudio de estabilidad de dos lotes de VC638 numerados 32 y 33, crecidos en fermentador de 5L (3L efectivos), el pellet fue resuspendido en una formulación a base de sacarosa, leche descremada y ácido ascórbico y secado en liofilizadora por solo 5 horas, se evalúa la estabilidad en el tiempo a dos temperaturas 5C y 25C usando como variable la viabilidad del cultivo después del secado. El procesamiento de los datos se realizó en el programa Graphpad Prism. Los resultados sugieren que esta variante de secado pudiera ser una opción dentro del proceso de desarrollo tecnológico que permita estabilizar el candidato vacunal a estas temperaturas lo cual representaría una ventaja considerable en el mantenimiento de la cadena de frío.

Key words: Stability test, VC638, vaccine, Vibrio cholerae
EIN-P-012  Enhancement of the nutritional quality of flour foliage Stizolobium niveum (Mucuna) using solid state fermentation with the fungus Trichoderma viride M5-2

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The use of alternative raw materials in the manufacture of animal feed to replace imports, reduces competitiveness with human food and preserves the environment, which is a challenge at present. The potential of legume foliage for its use as animal nutrition has been demonstrated. However, these meals have limitations from the point of view of bioavailability of several nutrients which could be attenuated by application of fermentation processes. Accordingly, through a solid state fermentation (SSF) biochemical and structural changes in the flour to remove many of these anti-nutritional components with a consequent increase in the nutritional value of the resulting product can be achieved. In this work the kinetics of the process of solid state fermentation of legume flour Stizolobium niveum with the fungus Trichoderma viride M5-2 is determined. The results showed that the 1-4 exo glucanase activity and pH have similar behaviors in inoculum sizes of 10^7 and 5*10^7 spores/g of dry S. niveum, reaching the cellulolytic activity its peak between 48 and 72 hours fermentation, while the pH always remained above 7. It was obtained a reduction of the neutral detergent fiber (NDF) from 24 hours and the greatest decrease (6, 14 percentage units) in the range of 48 and 96 hours of fermentation was obtained, indicating an increase in the nutritional quality of the fodder flour.

Key words: nutritional quality, Stizolobium niveum (Mucuna), solid state fermentation, fungus, Trichoderma viride M5-2
La marchitez bacteriana, causada por el complejo de especies de Ralstonia solanacearum, constituye una seria amenaza para la agricultura debido a su distribución mundial y amplio rango de hospedantes, por este motivo también han adquirido notable importancia los estudios para la caracterización de los aislados de este patógeno. El gen de la endoglucanasa (egl) de R. solanacearum ha mostrado presentar un elevado grado de conservación a nivel de especie y a la vez una buena resolución a nivel intraespecífico. Esto ha permitido dividir al complejo de especies en cuatro grandes grupos que se corresponden con el origen geográfico primario de cada aislado y se han podido realizar estudios de epidemiología molecular para establecer las relaciones existentes entre aislados de una región. Por tanto el objetivo de este trabajo es optimizar la amplificación por PCR del gen egl. El protocolo de amplificación por PCR se optimizó con la combinación del uso de herramientas informáticas y la estandarización in vitro de los principales parámetros de la reacción. Como resultado se obtuvo un programa de

**Key words:** optimización de pcr, egl, r. solanacearum
El cocobacilo Gram-negativo Bordetella pertussis es el agente causal de la tos ferina, enfermedad que ha sido controlada mediante vacunas celulares. Estas vacunas presentan el inconveniente de provocar reacciones adversas que aumentan con el número de dosis y la edad, por lo que se han ido desarrollando vacunas acelulares menos reactogénicas. Cuba solo cuenta con la variante celular de estas vacunas, por tanto cada vez se hace más necesaria la obtención de antígenos de B. pertussis para la sustitución de esta variante por una acelular. El principal factor de virulencia de B. pertussis es la toxina pertústica que inactivada constituye el componente principal de las vacunas acelulares anti-tos ferina. El presente trabajo describe los procedimientos realizados para la obtención de cepas de B. pertussis sobreproductoras de la variante S1-9K/129G de la toxina pertúsica atenuada genéticamente. Para lograr esto, fue introducida la cepa mutante LPA3-1-1 una segunda copia del operón que codifica al toxoide en el gen dnt, codificador de la toxina demonecrótica. Los mutantes obtenidos mantuvieron la capacidad de expresar otros antígenos como la Prn y la FHA y conservaron la identidad bioquímica, cultural y morfológica de la cepa LPA3-1-1 que les dio origen. Además, no se detectó actividad demonecrótica de un extracto celular proveniente de un cultivo en medio SS. El mutante d7 expresó niveles de toxoide significativamente superiores por lo que pudiera ser útil como cepa para la producción de vacunas anti-tos ferina tanto celulares como acelulares.

**Key words:** Bordetella pertussis, S1-9K / 129G, toxin genetically attenuated
EIN-P-015 Combined treatment with Growth Hormone-Releasing Peptide-6 and Epidermal Growth Factor counteracts aminoglycoside-induced ototoxicity and nephrotoxicity

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Ototoxic and nephrotoxic side-effects of aminoglycosides restrict their use in clinical practice. Combined treatment with growth hormone-releasing peptide-6 (GHRP6) and epidermal growth factor (EGF) has proven cytoprotective and antiapoptotic effects in other settings. The objective of this work was to study the effect of EGF-GHRP6 combination over cochlear and renal structures during aminoglycoside induced toxicity. Forty-four Wistar rats were assigned to five groups and submitted to treatments for 20 days. Control group was given PBS; the other groups received kanamycin. Treatment groups were concomitantly administered EGF-GHRP6 combination or the individual agents EGF or GHRP6. Eight weeks after treatments, cochleae and kidneys were studied by Light Microscopy. Cochleae were analyzed using semithin sections of resin-embedded cochleae. Histological condition of the organ of Corti and presence of cochlear hair cells were scored. Peripheral processes and spiral ganglion neuronal densities, and neuronal area were calculated. In paraffin embedded-renal cortex sections, the percent of damaged glomeruli and tubules was quantified. The density of preserved tubules and the presence of inflammatory cells or fibrosis were also estimated. The combined administration of EGF and GHRP6 preserved cochlear and renal structures simultaneously. It was related to the preferential individual effects of GHRP6 over cochlear structures, and EGF over renal epithelia. The lack of toxicity of these molecules, previously demonstrated in clinical practice, supports their possible use against the ototoxic and nephrotoxic effects of aminoglycosides. The advantages of EGF-GHRP6 combination validates the importance of developing combined therapeutic approaches in which the independent effects of its components are enhanced.

Key words: aminoglycosides, ototoxicity, nephrotoxicity, light microscopy, ghrp6, egf, egf-ghrp6
EIN-P-016  Insight of immune camouflage of the Human Papillomavirus types 16, 18, 31, 33 and 35: first steps for a final large-scale immunomic analysis, "the war go on"

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Introduction: Human papillomavirus (HPVs) is one of the most common causes of sexually transmitted diseases in both men and women around the world. Twelve HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are defined by the World Health Organization (WHO) as being high-risk cancer causing types, with additional types (68, 73) being recognized as 'possibly' cancer-causing. The greatest risk factor for the development of cervical and other cancers that have been linked to the HPVs family is the persistence of the virus. To persist for the decades required to develop HPV-related cancers, the virus must escape host immunity. The aim of this study was to discover by using a combined approach of immunoinformatics tools and statistical methods, possible proteins that allow HPV to evade the host immunity by analysis of the relative frequencies (epitope densities) computed from its aminoacidic sequences. Conclusions: The differences of epitopes densities found in all studied HPV proteomes suggest that HPV E5 plays a critical role in immune evasion, which is in correspondence with the experimental results reported to date. The combined approach of immunoinformatics tools and statistical methods can be used for a large-scale immunomic analysis to study viral proteins that modulate the major histocompatibility complex (MHC) class I molecules.

Key words: Human Papillomavirus, types 16, 18, 31, 33 and 35 large-scale immunomic analysis
EIN-P-017  Environmental performance of vaccine strain Vibrio cholerae 638: in vitro modeling

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The attenuated strain Vibrio cholerae 638 (O1, El Tor, Ogawa) is the active ingredient of the oral vaccine candidate CV638, which has proven to be well tolerated and immunogenic in several studies in healthy volunteers. Since this is a vaccine that manages the live attenuated biological agent, this is excreted in the feces of vaccine recipients. Then, in the regulatory environment, there are concerns about its potential environmental impact and the possibility of reversion to virulence. This work is an experimental approach to the possible performance of such strain on the environment. Survival of VC 638 suspended in water from different sources was assessed in several laboratory conditions. The results indicate the vulnerability of the strain to biotic and, it was also found that it does not displace wild strains under optimal growth conditions or nutrient deficiency. Susceptibility of the strain is determined under stress conditions which included agents as chlorine and detergents. VC 638 was more sensitive than its parental what could limit its persistence in natural ecosystems. The antibiotic susceptibility experiments showed that VC 638, as well as its virulent parent is resistant to polymyxin B and sensitive to other first-line antibiotics for the treatment of cholera in Cuba. Likewise, it was also tested the resistance to horizontal gene transfer, concluding that VC 638, as well as toxigenic strains currently circulating in the Caribbean, is resistant to natural transformation in the presence of chitin. VC 638 transformation frequency is 103 times lower than that of its parent. Finally, VC 638 has no advantage to compete and survive in natural environments.

Key words: cholera vaccine, vibrio cholerae, attenuated strains, environmental persistence
Introduction: The use of morphometrical tools in biomedical research permits accurate comparison of specimens submitted to different conditions. The surface density of structures are commonly used for this purpose. The traditional point-counting-method is reliable but time consuming and computer aided methods have been proposed. The objective of the present study is to compare the results of penile corpus cavernosum smooth muscle surface density in different groups of rats, as measured by the point-counting-method or by the color-based segmentation method. Materials and methods: Ten normotensive and 10 hypertensive male rats were used in this study. The rats’ penises were processed for obtaining smooth muscle immunostained histological slices. Photomicrographs captured from these slices were used for the analysis. The smooth muscle surface density was measured in both groups by the point-counting-method and by the color-based segmentation method. Results and discussion: The hypertensive rats presented an increase of smooth muscle surface density, as measured by the two methods. This may be interpreted as both methods being able to show the histological differences. However, the results on the hypertensive group were higher when measured by the point-counting-method. Thus, one should not use different methods for comparisons as they may give different results. Conclusion: The use of either method did not influence the final interpretation of the results. However, results obtained by one method should not be compared to results obtained by the other, as difference was found among them.

Key words: penis; morphometry, quantification, point-counting-method, color-based segmentation method
EIN-P-019 Escherichia coli diagnosis made in Huambo General Hospital

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Un diagnóstico certero para las infecciones urinaria se efectúa mediante la identificación de bacteriuria significativa a través del urocultivo. Escherichia coli es el microorganismo que con mayor frecuencia ocasiona infecciones del tracto urinario. Se considera el responsable del 90 % de estas infecciones. El objetivo de este trabajo fue describir la frecuencia de Escherichia coli aisladas en pacientes con infecciones urinaria atendidos en el Hospital General de Huambo. Se realizó un estudio bacteriológico descriptivo de todos los aislamientos de bacterias siguiendo los parámetros de nombre, edades, sexo y antecedentes de otras infecciones. Se obtuvo como resultado 114 cepas de Escherichia coli de pacientes con infección urinaria, el 84 (73,68 %) correspondió al sexo femenino y 30 (26,31 %) al sexo masculino, existiendo una diferencia estadística significativa entre ambas variables para su estudio epidemiológico. El 100 % de los pacientes atendidos en la provincia son de consulta externa.

Key words: Escherichia coli, diagnosis, Huambo General Hospital, Angola
EIN-P-020  Sensitivity and Resistance of Escherichia coli

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El diagnóstico etiológico de las infecciones urinaria se demuestra por los cultivos de una muestra de orina tomada adecuadamente en general revelan el agente causal de la infección, en concentraciones mayores de 100.000 unidades formadoras de colonias por ml (UFC/ml). El objetivo de este estudio fue determinar la sensibilidad y resistencia a los antimicrobianos de utilidad terapéutica de los aislamientos de Escherichiacoli identificados en los urocultivos de pacientes con ITU, atendidos en el Hospital central de Huambo. Se determina la susceptibilidad de los antimicrobianos a las cepas aisladas de los casos positivos a E. colise efectuó mediante la técnica estándar de difusión en agar de discos de papel impregnados en antibióticos, recomendación del CLSI 2013. La información que se generó se resumió en tablas estadísticas. Como resultado se obtuvo una buena sensibilidad con los antimicrobianos testados.

Key words: escherichiacoli, sensibilidad, resistencia
EIN-P-022Detection of a protein of Leptospira borgpetersenii with Immunogold and Immunohistochemistry

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Leptospirosis is considered the most widespread zoonosis in the world. In general, diagnosis is done either by laboratory techniques which detect antibodies against leptospiral antigens, or techniques such as PCR, immunofluorescence or immunohistochemistry that can detect either specific DNA sequences or surface antigens, respectively. Outer membrane proteins such as GspDL the Type 2 Secretion System (T2SS) secretin are expressed during infection and might be good targets for the immune response as well as good antigens that could be used for diagnostic purposes. The aim of this work was to detect whether GspDL is expressed during infection by means of an immunohistochemistry and immunogold assay. Hamsters (n=20), were inoculated IP with virulent Leptospira cultures (L. interrogans, serovar Canicola), previously isolated from dogs. The expression of GspDL was done in E. coli harboring the recombinant pAL208 plasmid, containing the L. borgpetersenii serovar Hardjo gspD gene (pET28a- gspD). Total proteins analysis was made in 12% polyacrilamide gels, followed by detection of the recombinant GspDL (rGspDL), with an anti-T7 peptide monoclonal antibody in Western blot assays. The rGspDL was purified by electro elution and finally, was used to produce a rabbit polyclonal anti-rGspDL immune serum. In situ detection of leptospires in kidneys and livers of infected hamsters was made with the polyclonal anti-rGspDL rabbit antibody by immunohistochemistry using the complex streptoiadine- biotin peroxidase. The results confirmed that GspD is expressed in hamsters during infection with L. interrogans serovar Canicola.

Key words: leptospira, t2ss, gspd, immunohistochemistry, immunogold
EIN-P-023    Expression of the L1 protein of the HPV in Escherichiacoli and hairy roots of broccoli

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The L1 protein of the human papilloma virus (HPV) has been used as antigen for developing vaccines, however the production level is still insufficient to meet the population needs. Plant molecular pharming technologies constitute a real alternative for production of L1 protein, and other therapeutic proteins. In this work, the production of L1 by hairy roots of broccoli with regard to Escherichia coli has been evaluated. Transformation of hairy roots was demonstrated by amplification of the cDNA and by the production of the L1 protein by immunohistochemical assays. The production of L1 in 4 strains of Escherichia coli DH5 was investigated. The immunohistochemical analysis revealed that the L1 protein was systematically expressed along the root tissue.

Key words: transgenic protein, heterologous protein, agrobacterium rhizogenes, pcambia, gusplus, broccoli
EIN-P-024 Expression and purification of human proinsulin from hairy root culture of Brassica oleracea var. italica (broccoli).

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The human proinsulin, the natural precursor of insulin, has been investigated as a neuroprotective and for the treatment of retinitis pigmentosa, acting on photoreceptor degeneration, synaptic connectivity, and functional activity of the retina. Human proinsulin is produced by recombinant bacterial systems, however the process is expensive. In this work we evaluated the expression levels of the human proinsulin in hairy root cultures of broccoli as an alternative model for production of this therapeutic protein. The expression levels and identity of the human proinsulin was evaluated in crude extracts and purified fractions of protein by SDS-PAGE and Western Blot techniques, respectively. Because of the small size of the proinsulin protein (9 kDa) it was purified by fractionation with 30 to 3 kDa molecular-cutoff membranes followed by molecular exclusion chromatography. The yield of proinsulin was 0.02% of total soluble protein determined by enzyme-linked immunosorbent assay (ELISA). The identity of proinsulin is being confirmed by MALDI-TOF analyses.

Key words: transgenic protein, heterologous protein, agrobacterium rhizogenes, pcambia, gusplus, broccoli
Modelling of biochemical processes for efficient enzymes production

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This research presents results of biochemical process modelling for enzymes production. The study addresses the field of biotechnology and offers a modelling tool based on the pseudo bond graph approach as base for process optimisation and conversion rate improvement. Superior performance regarding the operation of such processes can be achieved by applying modern techniques, both in terms of proper technologies and modelling methods. In this paper, a process for micro-organisms growth on two substrates that are produced by the hydrolysis of a primary complex organic substrate was widely analysed. More precisely, a three-step reaction network for lipase production from olive oil was investigated. A comparative analysis using numerical simulations of the biochemical process operated under two different experimental configurations is presented: the continuous and the batch operation modes, the study focusing on process mechanisms in transitory regimes. The influence of process parameters and reactants, as inputs, on reaction products formation was quantified along with process kinetics and its nonlinear characteristic. The time profiles of the primary and secondary substrates, enzyme and biomass concentrations are obtained and analysed, as well as the time evolution of dissolved oxygen and carbon dioxide concentrations. The obtained data reveal the importance of a reliable modelling tool for such nonlinear processes highlighted with respect to biochemical processes run optimizing.

Key words: biochemical process, lipase, modelling, simulation, process kinetics
EIN-P-026  Rapid non-enzymatic method for molecular typing of Saccharomyces cerevisiae by uni and two-dimensional pulsed field minigel electrophoresis

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The increasing incidence of multidrug-resistant microbial strains and its epidemic potential requires of ease access and better discriminative typing techniques to detect microorganisms with pathogenic markers. Pulsed Field Gel Electrophoresis (PFGE) has frequently been used for this purpose in both eukaryotic and prokaryotic cells. PFGE allows studying polymorphisms of a wide range-sizes DNA molecules, but small differences in molecules size are not discriminated in a single experiment. Two-dimensional PFGE allows to overcome such limitation, though it is cumbersome and costly. We propose a new procedure for the molecular subtyping of Saccharomyces cerevisiae strains using the PFGE technique in minigels (miniPFGE). It involves preparing PFGE samples using a single container (6 wells culture plate) for growing, harvesting and immobilizing cells of up to 6 strains, combined with a device to cut the samples with homogenous dimensions. Cell lysis was performed in situ, similar to a previously reported non-enzymatic method. Immobilized DNA was separated in miniCHEF or multi-miniTAFE chamber for one (1D) or two-dimensional (2D) PFGE, respectively. The electrophoretic patterns obtained using the proposed procedure were similar to those produced by non-enzymatic conventional methodology. However our proposal avoids multiple transfer steps, preserving the sample's physical integrity and preventing DNA degradation. 2D-miniPFGE allowed to analyze up to 12 strains simultaneously and to separate a larger number of bands, improving the PFGE technique resolution and discriminatory power. This procedure would be useful in the study and control of infectious diseases, as well as for monitoring the vaccination and immunization programs.

Key words: pulsed field gel electrophoresis; two-dimensional pfge; non-enzymatic method; subtyping;
EIN-P-027 Liposomal encapsulation of house dust mite allergens and dexamethasone modulates allergic response in a murine model of asthma

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Introduction: House dust mite (HDM) allergens are a major cause of asthma worldwide. HDM extracts or purified allergens are the current treatment of choice known as specific immunotherapy. The aim in such strategy is to drive immune reaction away from the allergic Th2 response by inducing Th1 and/or Treg cellular response. In that regard, Toll Like Receptor-activating adjuvants have been used for inducing Th1 response, but adjuvants capable of inducing the somehow safer Treg response are poorly investigated.

Materials and methods: we describe the anti-allergic properties, in a murine model of asthma, of HDM allergens Der s1 and Der s2 co-encapsulated with dexamethasone into dehydration-rehydration vesicles (DRV). Dexamethasone is a cortisol analogue with immune-suppressor activity known to induce specific Treg response, still with some adverse effects associated to systemic or prolonged use. Optimal lipid composition in terms of physical-chemical and anti-allergic properties was assessed using liposomes of cholesterol and different phosphatidyl-cholines (dipalmitoil-PC, distearoil-PC and egg-PC) encapsulating Der s allergens. Results: Although all liposomal compositions produced similar vesicle size, protein encapsulation and overall safe profile in treated mice, distearoil-PC:cholesterol liposomes produced significantly higher Th1-associated anti-Der s antibody levels before allergen challenge. Liposomes of this composition encapsulating dexamethasone were used to assess anti-allergic effects. Preliminary results indicate that encapsulation of HDM allergens and dexamethasone into liposomes diminishes allergic response traits such as IL-5 interleukin and IgG1 and IgE antibody levels. Conclusion: These results highlight the possibility of using delivery systems such as liposomes to modulate immune response and to prevent adverse reactions to free or soluble pharmacological compounds like dexamethasone.

Key words: liposomal vaccine, allergens, dexamethasone, anti-allergic response
EIN-P-028 Immune intrathecal response associated to dengue virus. Basis to the development of candidates vaccine

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Introducción: El virus del dengue es un virus ARN miembro de la familia Flavívidad. Recientemente en Cuba se han reportado pacientes con manifestaciones neurológicas asociadas al virus del dengue. Objetivo: Mostrar la respuesta neuroinmunológica asociada a patrones de síntesis intratecal de inmunoglobulinas mayores en seis pacientes cubanos con manifestaciones neurológicas asociadas al virus del dengue. Material y métodos: Se realizó un estudio retrospectivo en seis pacientes con manifestaciones neurológicas, con antecedentes de infección por virus del dengue. Los niveles de inmunoglobulinas mayores y albúmina se cuantificaron en suero y líquido cefalorraquídeo por inmunodifusión radial simple. La presencia de síntesis intratecal de IgM, IgG e IgA se demostró usando los reibergramas correspondientes. Resultados: Se observó el patrón de tres clases de inmunoglobulinas mayores, tanto en los pacientes con manifestaciones neurológicas postinfecciosas como en el transcurso del dengue. Un paciente desarrolló un Síndrome de Guillain Barré y mostró disfunción de la barrera sangre-liquido cefalorraquídeo sin síntesis intratecal de inmunoglobulinas mayores. Conclusiones: El conocimiento del proceso neuroinflamatorio y la respuesta inmune intratecal asociada al virus del dengue puede ser utilizada para el mejor conocimiento de la enfermedad y contribuir al desarrollo de posibles candidatos vacunales.

Key words: barrera sangre/liquido cefalorraquídeo, complicaciones neurológicas, dengue, reibergrama,
A novel tetravalent formulation combining the four aggregated domain III-capsid proteins, from dengue viruses, induces a functional immune response in mice and monkeys

Our group has developed a subunit vaccine candidate against dengue virus based on two different viral regions: the domain III of the envelope protein and the capsid protein. The novel chimeric protein from dengue-2 virus (domain III-capsid (DIIIC-2)), when presented as particulate aggregated incorporating oligodeoxynucleotides, induced antiviral and neutralizing antibodies, cellular immune response, and conferred significant protection to mice and monkeys. The rest of constructs were already obtained and properly characterized. Based on these evidences the present work was first directed to assess the immunological evaluation in mice of the chimeric proteins DIIIC of each serotype, as monovalent and tetravalent formulations. Here we demonstrated the immunogenicity of each protein in terms of humoral and cell-mediated immunity, without antigen competition on the mixture forming the formulation tetra DIIIC. Accordingly, significant protection was afforded as measured by the limited viral load in the murine encephalitis model. The assessment of the tetravalent formulation in non-human primates was also evaluated. In this animal model, it was demonstrated that the formulation induced neutralizing antibodies and memory cell-mediated immune response, with IFN-secreting and cytotoxic capacity, regardless the route of immunization used. Taken together we can assert that the tetravalent formulation of DIIIC proteins constitutes a promising vaccine candidate against dengue, and propose it for further efficacy experiments in monkeys against the four dengue serotypes.

Key words: dengue virus, tetravalent dengue vaccine, subunit vaccine
Evaluation of a sepharose CL-4B alternative, used in the immunoaffinity for obtaining the Cuban vaccine against hepatitis B

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Imunoaffinity chromatography is one of the purification techniques used for isolating of proteins for pharmaceutical or other applications. This technique is characterized by a high specificity, and ensures high product purity. One of the reasons for the rapid growth of the use of immunoaffinity chromatography has been the rapid advance in the field of biotechnology and molecular biology, as well as the need for purification of pharmacologically active proteins. In this sense, due to the need for high quality reagents and having difficulty for purchasing reagents for immunoaffinity chromatography by biotechnology centers in Cuba, authors sought assessing an alternative to Sepharose CL-4B obtained from Applichem (Germany), Applipurose CL-4B in the immunoaffinity chromatography performed to purify the Hepatitis B surface antigen. The parameters compared in this study were active sites amount, coupling efficiency, ligand density, ligand leakage, infrared spectrum and particle-size.

Key words: sepharose CL-4B, immunoaffinity Cuban vaccine, hepatitis B
EIN-P-031 Detection of an immunologically important epitope in the active pharmaceutical ingredient of Heberbiovac vaccine

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The presence of an immunologically-relevant epitope comprised between amino acids 134 and 153 of Heberbiovac® HB vaccine active pharmaceutical ingredient (API) using an enzyme-linked immunosorbent assay (ELISA) based on CB.Hep-4 monoclonal antibody (mAb) was investigated in this study. To reach this main subject, the validation of CB.Hep-4 mAb quantification system was firstly performed. The previously established ELISA to quantify CB.Hep-4 mAb was characterized by short incubation times at high temperature, which is unusual because of poor protein stability under these conditions. The linear range and recovery ranged 3.1-50 ng mL-1 and 95.2-105.3%, respectively. Maximum intra-and inter-assay variation coefficients were 6.5% and 7.0%, respectively. Thus, CB.Hep-4 mAb was quantified with specificity, precision, accuracy, and without interferences with this immunoassay. Then, several consecutive samples of Heberbiovac® HB API were monitored with CB.Hep-4 mAb, to demonstrate the presence of an immunologically-relevant epitope in this recombinant protein for vaccination.

Key words: immunologically important epitope, pharmaceutical ingredient, Heberbiovac vaccine
EIN-P-032 Application of polymerase chain reaction in real time in the diagnosis of samples of animals suspected of having rabies

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Rabies is an acute and fatal viral encephalitis caused by a single stranded negative sense RNA virus belonging to the genus Lyssavirus of Rhabdoviridae family. Rabies is enzootic and is still a major problem in developing countries. With the introduction of newer molecular techniques, particularly Real time PCR, the specificity and sensitivity of diagnosis of rabies increased. The aim of this study was to develop and validate a real-time RT-PCR assay for the diagnosis of rabies in Cuba. The assay uses a fluorophores FAM TaqMan probes targeted to nucleoprotein, that is a conserved viral genomic region. The positive control was obtained from positives samples and was cloned in PGenT vector. The analytical sensitivity of the assay was determined using serial dilutions of positive control. The assay demonstrated a wide dynamic range between 10^-1 and 10^-8 standard RNA copies per reaction. Good reproducibility was also detected, with intra- and inter-assay coefficients of variation ranging from 0.13% to 2.23% and 0.26% to 1.92%, respectively. The assay detected successfully RNA samples (n = 25) positives for direct immuno fluorescence and conventional RT-PCR indicating that this assay has a high sensitivity. Other common RNA viruses were tested negative, indicating high specificity of the assay. The sensitivity, rapidity, reproducibility and specificity of the real-time RT-PCR assay do this method suitable for detection and quantitation of rabies virus.

Key words: polymerase chain reaction, animal rabies
Non-invasive methods for the diagnosis of Helicobacter pylori are a useful alternative in clinical and epidemiological studies. Due to the lack of standardized methods using native antigens themselves or we set design of a strategy of extraction and obtaining sera immunoreactive antigens H. pylori infected patients. From the cultivation of H. pylori strains 196A (isolated from Cuban patients) and extracellular protein ATCC43504 and ultracentrifugation after the differential cell fractions for analysis of the same fraction in SDS-PAGE was obtained. The extracts versus control sera of patients with and without H. pylori infection, confirmed by invasive methods for the detection of immunoreactive antigens were evaluated. The extraction method by ultracentrifugation optimized resolution and allowed to separate proteins according to their subcellular localization. The differential amounts of proteins were superior to the periplasm and extracellular fractions. It was found to recognize several proteins from the native H. pylori strain, whose molecular weights of 55kDa (extracellular fraction), 98, 71 and 19kDa (periplasm), 134kDa (cytoplasm) and 149kDa (outer membrane), not detected in fractions obtained from the reference strain. This investigation allowed the establishment of a methodology for obtaining antigenic cell fractions, especially periplasm fraction and the outer membrane where the most immunoreactive proteins, which may be used as antigenic mixtures cuban strains in the diagnosis of infection with H. pylori.

Key words: helicobacter pylori, diagnosis, antigens, methodology, ultracentrifugation, protein fractions
La nefropatía diabética es una causa común de enfermedad renal en etapa terminal y de aparición de defectos congénitos en la descendencia. El objetivo de este trabajo fue describir el efecto de la vitamina E sobre la morfología renal de ratas diabéticas durante la gestación. Se desarrolló un modelo de nefropatía diabética inducida por estreptozotocina en ratas Wistar preñadas. La eutanasia se practicó el día 20 de la gestación. Los riñones se incluyeron en parafina para el estudio mediante microscopía óptica. Se midió el área glomerular utilizando el programa morfométrico Image J y se determinó el volumen glomerular. Se determinó el daño tubular proximal mediante un método semicuantitativo. El estudio ultraestructural se realizó en un microscopio electrónico de transmisión a partir de riñones incluidos en resina Spurr. En el grupo Control negativo se observó la estructura normal de la corteza renal. En las ratas diabéticas los glomérulos presentaron aumento de tamaño por incremento disfuso del mesangio, la membrana basal glomerular presentó engrosamiento irregular discontinuo y zonas de fusión de los pedicelos. Se observaron zonas extensas de túbulos atróficos. En el grupo tratado con vitamina E se observó menor ocurrencia de las lesiones glomerulares inducidas por la diabetes mellitus, con disminución del volumen glomerular hasta valores próximos a los del grupo Control negativo. En la mayoría de los túbulos proximales se constató la estructura normal, sin alcanzar los valores del grupo Control negativo. Este estudio demostró que la vitamina E ejerció efecto protector sobre la morfología renal de ratas preñadas.

**Key words:** vitamina e, nefropatía diabética, gestación, morfometría, ultraestructura, microscopía
The antimicrobial resistance using the diramic method in the Dr. ANTONIO LUACES IRAOLA hospital. A research during one years

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The Escherichia coli, Klebsiella pneumoneae and Proteus mirabilis have ar common. The resistance to multiple antibiotic in the Dr. Antonio Luaces Iraola hospital in Ciego de Avila province, the goal of this investigation was to determine the antimicrobial resistance with different antibiotics that was tested with the DIRAMIC method. A descriptive observational investigation was made about the bacterial resistance on the isolate piece in the nosocomial sepsis in the intensive care unit, we studied the 38 microbiological studies realized in the period between October 2012 and October 2013. We used the estándar Word method. The culture of the blood from catheter’s tip and from the surgical wounds were the most pathological products obtained. Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were isolated from the cultures and showed over the 40% resistance facing the tested antibiotics. In more than 90% of the tested antimicrobial were found resistance levels. the 90% of the pieces of Escherichia coli was resistance to Cefazolina and Sulfaprin. The 100% of the pieces of Staphylococcus aureus was resistance levels facing vancomicina. Acinetobacter showed resistance levels facing Carbapenemicos. The DIRAMIC method proved to be a fast and efficacious method to determine bacterial resistance.

Key words: bacterial resistance
EIN-P-036 Alpaca single domain antibodies (VHHs) as a tool for fascioliasis diagnosis and treatment

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Introduction: Human fascioliasis is a serious public health problem, with 2.4 million to 17 million infected people worldwide. Innovative new molecules are urgently needed to improve diagnosis and treatment of this infection, such as Alpaca VHHs generated against F. hepatica relevant antigens. Objective: The aim of the present research was to explore the use of VHH technology to produce specific and high affinity VHHs against F. hepatica excretory secretory (E/S) products.

Methods: Variable domain of heavy chain immunoglobulins (VHH) were amplified from PBMCs cDNA of E/S immunized alpacas. PCR products were inserted into a phagemid vector, next to the PIII protein sequence of a bacteriophage and transformed into E. coli TG1 in order to generate a representative VHH library. Phages displaying specific antibodies were selected by biopanning and clones recognizing Fasciola E/S antigens were tested by phage-ELISA using anti-M13 antibody. Each positive clone was sequenced in order to identify different variants.

Results: A library with $1 \times 10^7$ clones was generated and phage displayed. After three successive biopanning steps, 50 E/S-specific VHHs were identified by phage-ELISA and specific VHHs fragments size ranged 400-600bp. Sequence analysis of each clone revealed 27 different sequences.

Conclusion: The results evidence the potential of VHHs to generate new specific molecules that could be used to develop new tools for diagnosis and treatment of fascioliasis.

Key words: Alpaca single domain antibodies, (VHHs), fascioliasis diagnosis, treatment
Combination of nutritive bases and selective inhibitors in a chromogenic composition for gram-positive cocci.

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The reemergence of infections caused by gram-positive shows an increasing of their pathogenic potential. The fast and accurate microbiological diagnosis of these agents gains significant clinical importance. In BioCen was developed a chromogenic composition for the isolation, culture and rapid differentiation of Gram-positive bacteria through specific chromogenic reactions in which Gram-negative bacteria are partially or fully inhibited. To develop a chromogenic composition for the isolation and differentiation of clinical relevant species of the genera Enterococcus, Streptococcus and Staphylococcus with an appropriate mixture of nutritive bases and selective inhibitors in the formulation. As biological material a total of 45 microbial strains ATCC were tested and isolated from clinical specimens of the genera Streptococcus, Enterococcus and Staphylococcus and other gram-negative organisms. Different concentrations of selective inhibitors for Gram-negative bacteria and nutritive bases, combined to promote the growth of Gram-positive bacteria were evaluated. Microbiological functionality of the final chromogenic composition was evaluated and was determined a series of quality diagnostic parameters. Selective inhibitors suppressed gram-negative organisms completely, except Proteus mirabilis and Pseudomonas aeruginosa. The combination of nutritive bases allowed the differentiation of fastidious Gram-positive through specific chromogenic reactions and in only 24 hour. In average, parameters such as sensitivity, specificity and diagnostic accuracy were satisfactory. The chromogenic composition obtained allows the isolation and differentiation of species of the genera Enterococcus, Streptococcus and Staphylococcus clinical significance.

Key words: gram-positive organisms, gram-negative organisms, nutritive bases, selective inhibitors,
EIN-P-038 Nutritional and selective capacity of a chromogenic and fluorogenic diagnostician for salmonella

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Salmonella is the causative agent of some foodborne diseases and typhoid fever, reporting high rates of morbidity and mortality worldwide. Nutritional and selective capacity of a new chromogenic and fluorogenic diagnostic device for different serotypes of Salmonella of clinical and public health significance was evaluated. 52 Salmonella strains and a representation of other bacteria and yeasts were selected. Nutritional capacity of composition was determined by spectrophotometric technique using variants with mixtures of nutritive bases, recording the increase in biomass. Nutritional capacity and inhibition of two experimental variants with different inhibitors were tested, by determination of quantitative parameters. The productivity of the final formulation was compared with a reference medium (Oxoid, England). Salmonella strains showed plenty of growth in the variants made with different nutrient combinations. Both experimental formulations showed their ability to recover and inhibit microorganisms of interest. The final composition showed productivity values for: Salmonella ser. Typhimurium ATCC 14028 0.84±0.2; Salmonella ser. Typhimurium ATCC 13311 0.90±0.1; Salmonella ser. Typhi ATCC 19430 1.12±0.1; Salmonella ser. Typhi ATCC 7259 0.96±0.1; Salmonella ser. Enteritidis ATCC 13076 0.47±0.1; Salmonella ser. Paratyphi A ATCC 8005 1.08±0.1. The new chromogenic and fluorogenic diagnostic device is able to promote the growth of Salmonella and inhibit other bacteria and yeasts, with appropriate productivity and selectivity values according to ISO 11133, for the selective media.

Key words: cromocen salm culture medium, salmonella, diagnostician, productivity, selectivity, nutrient
EIN-P-039 Study of recovery and differentiation of aeromonas.spp with different diagnosticians

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At the present time the species of the Aeromonas genus has emerged as a public health problem for the world population. The increase of the isolations of Aeromonas species from water and food samples, have driven to deepen in the study of the pathogenicity of this genus. Increased of the recovery capacity and differentiation of Aeromonas species using CromoCen AE and CromoCen AGN media was studied. Certified strains of Aeromonas, Staphylococcus, Enterococcus and Streptococcus were used as biological material. An experimental design was used with different concentrations and combinations of inhibitory agents for Gram positive bacteria. The recovery capacity of CromoCen AE medium, was evaluated vs CromoCen AGN medium, relating the recounts between both culture media. The color developed in the colonies by the degradation of the chromogenic substrate was observed in the different species of evaluated Aeromonas. The reached concentration of 1 g/L between the inhibitors sulfite and sodium desoxicolate in the CromoCen AE media, allowed the inhibition of the tested strains of Enterococcus, Streptococcus and Staphylococcus in a 10-2 dilution. The combination of chromogenic substrate for detecting -galactosidase activity facilitated the differentiation of Aeromonas species. A chromogenic medium intended for diagnostic, that allow a higher recovery and differentiation of the Aeromonas species and also to inhibit gram positive microorganisms, was obtained.

Key words: recovery, aeromonas, chromogenic agents inhibitors
The study of the variability of the incubation time, culture temperature and sample volume to be inoculated determined the reliability of using CromoCen CND-C as a chromogenic-fluorogenic method for the identification and detection of different Candida species. The aim of this study was to evaluate the robustness of the method studying the influence of various factors on performance. A suspension of Candida albicans was used with a DO580 of 0.250-0.280 to inoculate the plates prepared with Sabouraud Dextrose Agar and CromoCen CND-C. The robustness of the temperature was studied selecting the values of 28, 35 and 42°C for 48 h of culture. Periods of 36, 48 and 72 h of incubation were tested. The linearity was evaluated with inoculum volumes: 0.05; 0.1; 0.2 and 0.4 mL at 35°C for 48 h. The enumeration of colonies per plate allowed calculating the uncertainty of measurements in each assay. The robustness of the method was demonstrated in all the range from 28 to 42°C. Consistency of the counts in the intervals between 36 and 48 h, and from 36 to 72 h was not observed, whereas robustness was found in the period between 48 and 72 h. The index of proportionality of the reference method and the alternative method showed that the procedures guarantee the overall linearity of results. We conclude that the chromogenic method with the use of CromoCen CND-C is robust and it ensures the reliability of the results.

**Key words:** reliability, robustness, linearity, measurement uncertainty, candida, chromogenic and
EIN-P-041 Functional assessment of aerobic hemocen diagnostician during accelerated stability study

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Introduction: Bacteremia is a serious complication of bacterial infections. Early diagnosis of the causative organism allows the appropriate treatments in a shorter time interval. HemoCen Aerobic is a diagnostic device designed for this purpose. Objective: To evaluate the functionality of a pilot batch of Aerobic HemoCen during accelerated stability study. Materials and Methods: A pilot batch of HemoCen Aerobic was formulated and aseptically packaged at the National Center of Bioproducts and at the Biological Pharmaceutical Laboratories, LABIOFAM. The accelerated stability study was carried out following Arrenhius Method. The bottles were stored for 120 days at 15 °C, 30 °C and 50 °C. The physicochemical, organoleptic and growth promotion capacity evaluation of Staphylococcus aureus ATCC 25923 was carried out at the 7, 15, 30, 60 and 120 days. Results: The stability study demonstrated that the pH of the medium and the color deteriorate progressively with increasing temperatures over time. Growth promotion of Staphylococcus aureus during the study was favorable with recovery rates between 2 and 4 CFU/mL of suspension. The statistical processing yielded an estimate of durability of two years. Conclusion: The functionality of the pilot batch of HemoCen Aerobic diagnostician during accelerated stability study was successful with recovery rates of Staphylococcus aureus between 2 and 4 CFU/mL of microbial suspension, with a period of 2 years of durability estimation. The pH and color deteriorate progressively over time, when the temperature increases between 30 and 50 °C.

Key words: hemocen, diagnostician, functional, stability study, growth promotion, temperature
Clinical performance evaluation of hemocen aerobic a new hemoculture medium

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The rapid detection of microorganisms in blood samples is still a valuable solution for the adequate and timely antibacterial therapy. It was carried out a comparative study of the HemoCen Aerobic vs. a reference commercial medium (Liofichem, Italia) according to ISO 16140 (2003) norm in 82 positive samples and in 21 negative samples (ages between 17 and 85 years). Two samples were contaminated (1,9%) with coagulase negative Staphylococcus. Other 22 samples were true positive (21,8%); 76 true negative (75,2%) y two false negative (2%). Among the lasts, one did not show growth in HemoCen, but in the reference medium grew and yeasts were confirmed. The same result was obtained in "Hospital Clínico - Quirúrgico Hermanos Ameijeiras". The simple with a positive yeast growth in HemoCen Aerobic, as well as 4 samples with positive growth of Staphylococcus aureus, did not showed positive reaction in reference medium. The detection of Gram positive bacilli was coincident with the reported results (50% of the bacteremia). The diagnostic sensibility reach 91,6%, diagnostic specificity - 98,7% and diagnostic accuracy - 97,0%. The percentage of positivity (20,7) fall in the range of results reported previously (from 14% to 37,8%). It was possible to isolate a broad spectrum of microorganisms such as Staphylococcus aureus (50,0%), Escherichia coli (13,6%), yeasts (4,5%), Acinetobacter spp. (9,0%), Klebsiella pneumoniae (9,0%), Serratia marcescens (9,0%) and Acinetobacter calcoacetium anitratum (4,5%).

Key words: hemoculture, diagnostic parameters, comparative study
EIN-P-043   Safety of Racotumomab in the treatment of patients with non-small cell lung cancer

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In Cuba, lung cancer is the second cancer in incidence and the first in mortality. Therefore, it is necessary to identify new therapeutic options. Immunological approaches are interesting because of the potential activity without the toxicities of conventional chemotherapy. Center of Molecular Immunology generated a vaccine called Racotumomab; it acts on the lung carcinoma observing an increase in tumor apoptosis and a decrease in the number of tumor vessels. In order to assess its safety an expanded access, multicenter, open study was conducted in 86 patients with non-small cell lung cancer. The dose administered was 1mg/mL by intradermal route. The first 5 doses were administered every 14 days and the remaining 10 each 28 until to complete treatment. The follow up reinmunizaciones was every 28 days. The occurrence of adverse events (AE) was analyzed and they were classified according to CTC criteria v4.02. The 67.4% (58 patients) reported EA for a total of 215 events. The most frequent was burning at the injection site 32 (14.9%). The use of the vaccine in the patients studied showed good safety and tolerance.

Key words: racotumomab, clinical trial, non-small cell lung cancer, adverse events
EIN-P-044 Cold chain: distribution of the investigation new drug of clinical trial.

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Logistics vaccine was introduced in the 80’s through the Expanded Programme on Immunization (EPI) promoted by WHO and PAHO. In 1990, Technical Network for Logistic in Health (TECHNET) joined to EPI. TECHNET was consisting of experts in health logistics, in order to establish global technical guidelines for planning and management logistics systems related with immunization programs. Due to the lability characteristics of vaccines, is essential reducing the factors that can decrease their quality. Given the increased number of global regulatory and standards-based guidance documents issued over the last years, members of the pharmaceutical supply chain are taking notice and making changes to ensure product quality and protect patient safety. To comply with it, a practical manual was designed in order to ensure the optimal use of the investigation new drug for clinical trials. Journal articles, monographs from Internet and books from the regulatory agencies as: FDA, EMEA and CECMED, were reviewed. The manual includes the resources, methods and materials necessary for the control of the cold chain. So, it is a valuable tool for all professionals involved in the storage, handling and distribution of cold storage products, because the success and quality of the research are strongly related to the degree of compliance the assumptions set out in this document. The management of temperature-sensitive drugs represents an increasingly important component of the global pharmaceutical supply chain. Therefore, this manual can be used for all centers from the Scientific Polo that produce, research and marketing this kind of drugs.

Key words: cold chain, clinical trial, vaccine, investigation new drug
Methicillin-resistant Staphylococcus aureus isolated from skin infection

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La resistencia bacteriana a los antibióticos trae consigo serias dificultades en el tratamiento de las infecciones, en particular Staphylococcus aureus resistente a meticilina (SARM) representa un serio problema de salud, ya que genera complicaciones terapéuticas en patologías que incluyen infecciones cutáneas. El presente trabajo se propuso como objetivo determinar la prevalencia de cepas SARM en lesiones de piel de pacientes hospitalizados. A partir de muestras de lesiones en piel de 78 pacientes ingresados en el Hospital General "Iván Portuondo", en el período 2011-2012, se realizó el aislamiento microbiano y la caracterización de los aislados por métodos convencionales. Se utilizó el método de Kirby-Bauer de acuerdo con las especificaciones de la CLSI sobre placas de agar Mueller-Hinton y pruebas de identificación y sensibilidad mediante el sistema automatizado Vitek 2Compact. De un total de 109 aislados bacterianos, 50 resultaron positivos para Staphylococcus aureus subsp. aureus, de los cuales el 84 % mostraron resistencia a 3 o más antibióticos entre los que se incluye cefoxitina para 38 de las cepas, las que presentaron 18 patrones de resistencia. La identificación de Staphylococcus aureus meticilina resistente fue ratificada por el sistema Vitek 2, al revelar la existencia de un 76% de prevalencia de SARM. Estos resultados indican la concordancia entre los métodos utilizados y la elevada incidencia de esta bacteria en las muestras analizadas.

Key words: staphylococcus aureus, resistencia, antibióticos, meticilina
DNA fingerprinting of microorganism by pulse field gel electrophoresis (PFGE) is a useful method to discriminate subtypes of pathogens. PFGE require to a method that allowing to prepare pure and intact isolating DNA molecules. Achieving DNA with such features requires expensive methods and also time consuming. In GUEFAST Laboratories of the Department of Molecular Biology of Neuroscience Center, two types of kits that allow obtaining immobilized and intact DNA yeast and bacteria, mainly consisting of salts and detergents are produced, making them less expensive without losing their effectiveness and require shorter treatment times. The purpose of this study is to demonstrate the useful life of Levkit and Backit used in the isolation and purification of genomic DNA from bacteria and yeast. A stability study was conducted by long-term. The parameters of interest at the start of production, a year, two years and five years of storage were included. The study included qualitative and chemical variables, and functional tests by PFGE. The results showed no significant changes in the ranges specified for the chemical composition or the organoleptic characteristics. The electrophoresis showed that over time storage kits were able to render equal amounts of DNA and showed identical electrophoretic patterns. It was concluded that the useful life of the kits stored at 4 °C is 5 years.

Key words: stability; dna fingerprinting ; dna preparation with kits ; pulsed-field minigel electrophoresis
EIN-P-047 Diagnosis of the urinary infection by the culture of the urine. Using the Diramic method in Dr. Antonio Luaces Iraola hospital. A research during two years

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The urinary infection suggest mioorganism’s presence inside the urinary system. It was done descriptive observational retrospective research about the diagnosis of urinary infection by Culture of the urine using the DIRMIC method in Ciego de Ávila’s hospital. The urinease and the simple were all of the culture of the urine received in the microbiology laboratory in the period from May 2009 to May 2011. The results obtained in the urine’s culture were analyzed. It let us know the isolation frequency of the etiological agents in the infection of the urinary tract and it was determined the resistance patterns of the microorganism vs the different tried antimicrobial. The highest positivisms percent was obtained in patients percent from external office. The no useful samples percent was in adequate parameters. There were low resistance rate facing “acido nalidixico” and “nitrofurantoina” but on the contrary, it was quite different facing Sulfaprin whose resistance rate was high. Escherichia coli was the microorganism with the highest isolation frequency, followed by Pseudomonas aeruginosa, Proteus vulgaris and Proteus mirabilis. The use of DIRAMIC method was an advantageous method in the determination of the urinary tract’s infection.

Key words: infection, diramic
Nanotechnology should play a decisive role in resolving the current the main problems of the diagnostic microbiology: speed of detection and accuracy. In Cuba different nanocomposites have been developed, based on the fast fluorogenic identification of several microorganisms. The present study was directed to characterize a new nanocomposite based on tricalcium phosphate salt nanostructure combined with a highly nutritive composition and with a fluorogenic dye derived from methylumbellipherone. The proper nanostructure, as well as the nanocomposites were characterized by TEM, EDS, FTIR, TGA; zeta potential, particle size, pH and antimicrobial activity were also tested. The influence of the sample volume, inoculum concentration, hydration volume, and the detection method (visual and spectrophotometric under UV light) were tested over the incubation time. E. coli was tested as target microorganism. It was demonstrated that nanostructure did not inhibit bacteria growth. It was possible to detect E. coli in a suspension at a concentration of $3 \times 10^8$ CFU/mL, with only 0.1 g of the nanocomposite, in a sample volume of 0.4 mL and a minimum incubation period of 1:30 h when observing the reaction visually and in 1 h in an spectrophotometer under UV light at 365 nm.

Key words: nanocomposite, e. coli, fluorogenic detection
EIN-P-049  Producción de la hormona del crecimiento humano (hGH1) en cultivos de raíces transformadas de Brassica oleracea var. italica

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Plant cell, tissue and organ culture based expression systems have been investigated as an alternative for large scale production of therapeutic proteins. In this work hairy root cultures of Brassica oleracea var. italica (broccoli) were established to produce the human growth hormone (hGH1). The hGH1 cDNA was cloned into the pCambia1105.1 binary vector to induce hairy roots in hypocotyls of broccoli plantlets via Agrobacterium rhizogenes. Most of the infected plantlets (90%) showed hairy roots when infected before appearance of the true leaves, and unlike conventional hairy root induction protocols, keeping the emerging roots attached to hypocotyl explants transferred to solid SH medium culture significantly improved the proliferation of homogeneous lines of transformed roots. The presence and expression of the cDNA in the hairy root cultures was confirmed by PCR amplification from genomic DNA and western blot from semi-purified protein extracts respectively. The hGH1 quantified by ELISA yielded 0.2–10 µg/g DW*d, with a productivity of 0.003–0.642 µg hGH/g DW*d. Twelve hGH1-producing hairy root lines retained their ability to produce hGH1 after a period of 16 months. Although these results varied among the transformed lines they were consistent with those reported for other heterologous proteins produced with similar plant expression systems, and suggest that further screening of hGH1-producing hairy roots cultures could represent a real opportunity for large scale production of hGH1.

Key words: somatotropin, heterologous protein, agrobacterium rhizogenes, pcambia, gusplus, broccoli