COVER PAGE FOR POST-KATRINA SUPPORT FUND INITIATIVE
PRIMARILY RESEARCH SUBPROGRAM PROPOSALS
BOARD OF REGENTS SUPPORT FUND, FY 2006-07

008PKSFI-R-07

1. Submission Discipline: (check only one) ☑ Biological Sciences ☐ Information Technology ☐ Materials Science	(For BoR Use Only) Application Number:
2. Name(s) of Lead Submitting Institution of Higher Education: (Include Branch/Campus/Other Components) LSU Health Science Center – New Orleans	
3. Address of Lead Institution of Higher Education: (Include Dept/Unit, Street Address/P.O. Box Number, City, State, Zip Code) 433 Bolivar St, New Orleans LA 70112	
4. Title of Proposed Project: Center of Excellence for Vaccine Development	
5. Proposed Duration: (Circle # of Yrs.) 1 2 3 4 (5)	6. Funds Requested P-KSFI Year 1: $1,298,861 ESIP: $601,262 Project Total: $7,212,558
7. Name(s) of Partnering Institution(s) Tulane University Health Sciences Center; Xavier University – New Orleans	
8. Does This Proposal Contain Confidential or Proprietary Information Which Falls Into a Category Described in R.S. 44:4(16)? ☐ YES ☑ NO (NOTE: If YES, the proposal MUST be appropriately marked.)	

By signing and submitting this proposal, the signators are certifying that: (1) the proposed project has not already been funded/is not currently being funded/has not been promised funding; (2) this proposal has been reviewed and approved by an Institutional Screening Committee; and (3) the institution and the proposed project are in compliance with all applicable Federal and State laws and regulations, including, but not limited to, the required certifications set forth in: (a) Grants for Research and Education in Science and Engineering, NSF Grant Proposals Guide (GPG), NSF 03-2, effective 10/1/02, and (b) 45CFR 620, Subpart F (Requirements for a Drug-Free Workplace).

Name/Title/Institution	Dept./E-mail address/Telephone Number	Signature
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Co-PI John Clements PhD Professor and Chairman, Dept. of Microbiology & Immunology,Tulane University Health Sciences Center	Dept Microbiology & Immunology, Tulane University Health Sciences Center, 504-988-5070, jclemen@tulane.edu	
Co-PI Seth Pincus MD, Nelson Ordway Professor of Pediatrics LSUHSC, Director, Research Institute for Children	Dept. of Pediatrics, LSU Health Sciences Center, 504-894-5376, spincu@lsuhsc.edu	
Co-PI Tarun Mandal PhD, McCaffrey Norwood Professor of Pharmacy, Xavier University	Dept Pharmaceutical Sciences, Xavier University, 504 520-7442, tmandal@xula.edu	
Co-PI		

Campus Head or Authorized Institutional Representative		Dean*		Authorized Fiscal Agent	
Name/Title: (type or print) Joseph M Moerschbaecher III		Name/Title: (type or print) Dr. Larry Hollier		Name/Title: (type or print) Ronnie Smith	
Signature:		Signature:		Signature:	
Date: 3/16/07	Telephone Number:	Date: 3/16/06	Telephone Number:	Date: 3/16/07	Telephone Number:

* If multiple deans from the lead institution are involved in project activities, the dean with authority over the primary submitting department will serve as signatory for all.

GOALS AND OBJECTIVES

This is a proposal to establish a Center of Excellence in Vaccine Development with an outstanding critical mass of participating investigators at several institutions in New Orleans that will encompass all stages of vaccine development. Major objectives are:

1. establish a framework for research and development in infectious disease vaccines that will provide a focus for retention of established researchers and promising junior scientists;
2. stimulate the recruitment of new scientific talent into the State; and foster new collaborations between scientific institutions in Louisiana;
3. in this way, aid in the overall recovery of the research and educational missions and infrastructure at three institutions that sustained damage in the aftermath of Hurricane Katrina;
4. develop research projects and related academic activities in vaccine development that underpin education and training of new generations of students and postdocs in Louisiana;
5. strengthen our capacity to compete for large-scale national and international research and development grants and contracts in vaccines, therapeutics and biodefense (eg. from the NIH and other US Government agencies, the Bill and Melinda Gates Foundation, and Industry);
6. develop local infrastructure necessary for the development and clinical testing of novel vaccines for infectious disease;
7. promote interactions between basic and clinical researchers in development, testing and application of novel vaccines and therapies for infectious disease in Louisiana and elsewhere;
8. develop intellectual property in the form of patents and licenses;
9. promote linkages with Industrial partners both locally and nationally and establish new commercial and biotechnology opportunities in Louisiana;
10. positively impact regional, national and international public health.

By the end of the first year our goal is to have each of the Cores fully functional for Center projects, including all new equipment, and research assistants in place and fully trained in usage of equipment and data collection and analysis.

By Yr 2 we will have new faculty appointed in vaccine research, 4 new interdisciplinary Center vaccine projects underway, at least 2 Center postdocs appointed to Center projects, multiple conference abstracts and manuscripts based on Center projects submitted, five new project grants submitted to NIH and/or other funding bodies with at least 1 new NIH or equivalent award and 1 industry-funded research grant awarded, five manuscripts accepted in high-medium impact journals, new summer students, graduate students and postdocs enrolled, 2 new patents submitted, at least 2 out-of-State meeting/major seminar invitations, meetings in place with Institutions/State/Industry concerning possible local dedicated GMP facility manufacture,

Year 4: as in Yrs 2 and 3, plus at least one P01 program and/or COBRE vaccine-related grant submitted to NIH or equivalent, at least 2 new junior faculty appointed with start-up commitment from Center and Institutions in new Institutional space, IND in preparation for at least two Center clinical trials, fundinglinkages established with industrial partners.

Year 5: as in Yr 4, plus IND in preparation for at least two Center clinical trials; award or resubmission of P01 and/or COBRE vaccine-related grant(s).

The long-term goal is to establish Louisiana as a center of excellence in vaccine research and development with appropriate infrastructure to support its future growth and expansion through large-scale national, international, and private funding, commercial and industrial partners, patents, licenses and royalties. The Center of Excellence in Vaccine Development would also provide a cornerstone for any future regional enterprise that could ultimately consolidate vaccines and therapeutics R&D in the South.

NARRATIVE AND BIBLIOGRAPHY

(a) Project Rationale and Structure

(1) DESCRIPTION OF RESEARCH GROUP

A major strength of the Center is the interdisciplinary nature of the science brought by the different institutions that can be focused towards solutions to specific problems in vaccine research. Each of the participating institutions brings complementary strengths, and consequently, investigators will ultimately have access to a group of Core technologies and research strategies not currently available at any single institution. These Cores, based on strong existing facilities, will be developed to support the flow of vaccine research and development for all participants.

Alistair Ramsay PhD will serve as Principal Investigator and Center Director. He has established expertise in vaccine immunology and played a key role in the development of the important prime-boost vaccination strategy. He holds NIH R01 Project and P01 Program funding in vaccine immunology and vaccine development against tuberculosis and HIV. He is currently Professor of Medicine and Director of the Gene Therapy Program at LSU Health Sciences Center. He is also Director of the Health Excellence Fund Center for Gene Therapy for Genetic and Acquired Disease, a 5-year Program funded by the Louisiana Board of Regents that co-ordinates seven interactive research projects within a multi-institutional consortium of PIs from local institutions, including LSU and Tulane University Health Science Centers.

John Clements PhD is co-PI and a member of the Center Steering Committee. He is Professor and Chairman of Microbiology & Immunology at Tulane University HSC. His research program is focused on development and delivery of vaccines against infectious diseases for which he has an international reputation. He is currently PI on two NIH grants and one Dept of Defense Grant and has been funded continuously from public and industrial sources for many years. Dr Clements also has significant expertise in biodefense, both in vaccine development and as a member of the Iraq Survey Group of the DOD, Baghdad, Iraq, and the United Nations Monitoring, Verification and Inspection Commission training program.

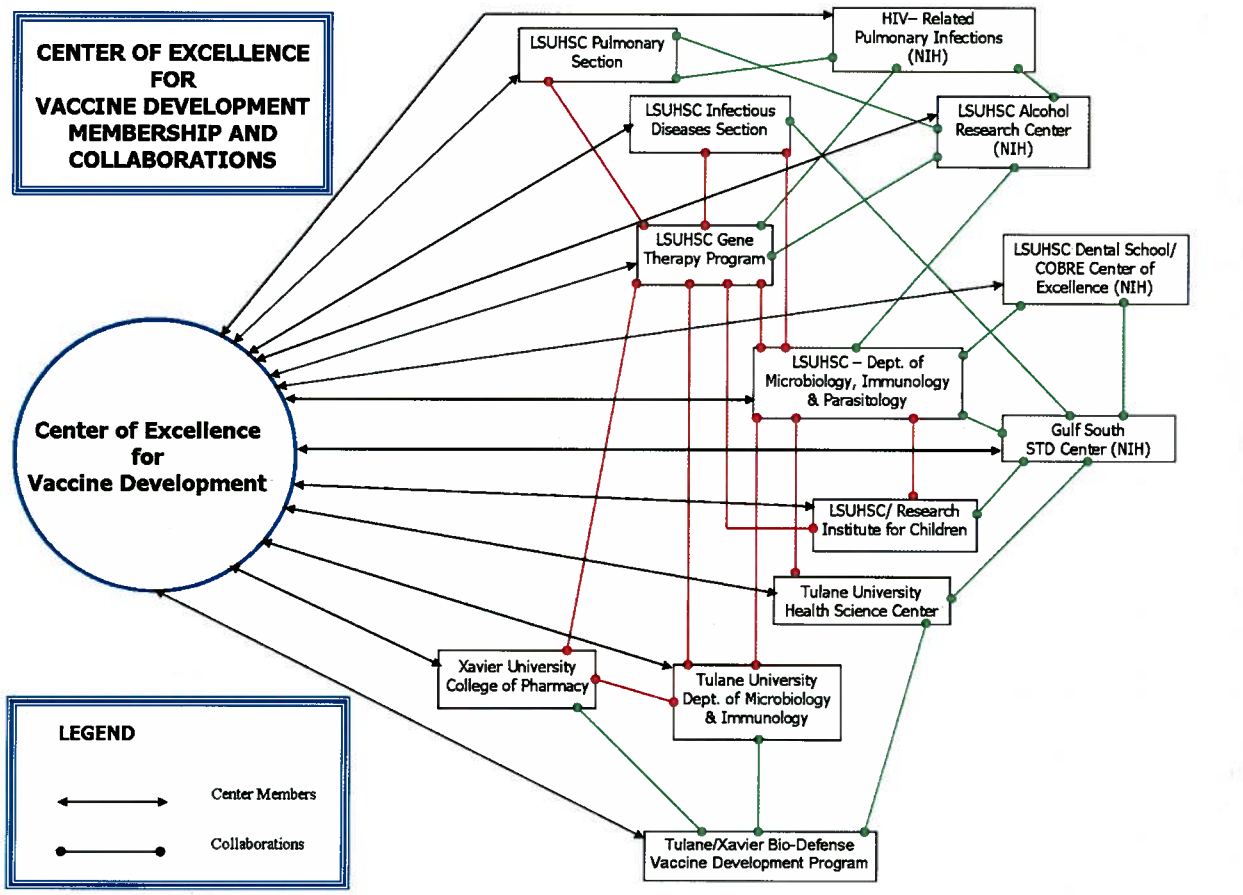
Seth Pincus MD is co-PI and a member of the Center Steering Committee. He is Nelson Ordway Professor of Pediatrics at LSU Health Sciences Center and Director of the Research Institute for Children in New Orleans. His NIH-funded research program is focused on interactions between antibodies and pathogenic agents, with current emphasis on HIV, group B streptococci and plant and bacterial toxins, including ricin. He is currently Head of the NIH AIDS Vaccine Study section (VACC, 2005-2007) and is a member of the Scientific Councillor's Review of the NCI Intramural AIDS Vaccine Program.

Tarun Mandal PhD is co-PI and a member of the Center Steering Committee. He is McCaffrey Norwood Professor of Pharmacology at Xavier University and has nationally recognized expertise in nanoparticle development and sustained release for delivery of drugs and biologicals for which he currently holds two NIH grants.

Other key investigators are: David Martin MD (Head, Infectious Diseases, LSUHSC) who directs the NIH-funded (U19) *Gulf South Sexually Transmitted Infections/Topical Microbicide Co-operative Research Center* that is focused on clinical research of host-pathogen interactions in *Chlamydia, M. genitalum* and *T. vaginalis* infections. He has N01 funding to support clinical studies of STDs through the HIV Outpatient (HOP) Clinic and the Delgado Personal Health Clinic in New Orleans at which he runs a world class diagnostic STD lab; Steve Nelson MD (Head, Pulmonary/Critical Care, LSUHSC) who studies effects of alcohol consumption on host defense with emphasis on HIV infection. He directs the NIH-funded (P60) Comprehensive Center: *Alcohol, HIV infection and host defense*; Judd Shellito MD (Prof. Medicine, LSUHSC) who is Program

Director of the NIH-funded P01 Program: *Host-defense & HIV-related pulmonary infection* and holds R01 funding for his work on host responses to bacterial pneumonia; Paul Fidel PhD (Prof. Microbiology, Immunology & Parasitology (MIP), Director, Center of Excellence for Oral & Craniofacial Biology, LSUHSC) who directs the NIH-funded (P20) Center of Biomedical Research Excellence (COBRE) Training Program: *Mentoring Oral Health in Louisiana* (oral infections) and an R01 in HIV-related immune dysfunction in oral candidiasis; James Robinson MD (Prof. Pediatrics, Tulane University Health Science Center) who is internationally recognized for his work on antibody responses to HIV envelope glycoproteins, particularly neutralizing antibodies. He holds NIH Program funding for this work through the Center for HIV/AIDS Vaccine Immunology (CHAVI) and has extensive support from the Gates Foundation: Global HIV/AIDS Vaccine Enterprise (GHAVE) and the Grand Challenges in Global Health-Foundation for the NIH (Bill & Melinda Gates Foundation); Jim Cutler PhD (Prof. Pediatrics, LSUHSC, Associate Director of Research Institute for Children) who is internationally recognized for his P01 and R01-fundedwork on host-pathogen interactions and identification of vaccine antigens in *Candida albicans*; Ronald Luftig PhD (Professor and Chair of Microbiology, Immunology & Parasitology (MIP), LSUHSC) who is funded for clinical/diagnostic research on hepatitis C virus in Central America and holds mentorship/advisory roles in the NIH-funded (P20) Center of Biomedical Research Excellence Training Program: *Mentoring Oral Health in Louisiana*; Vijay John DEngSci (Professor and Chair, Dept. Chemical and Biomolecular Engineering, Tulane) who has NIH and NSF funding for his work on lipid self-assembly and development of nano-structured materials; Scott Grayson PhD (Asst Prof. Dept. Chem. Biomolecular Engineering, Tulane) is supported by NIH for his work on multi-domain lipid-based nano-particles for improved vaccine delivery; Carol Mason MD (Prof. Medicine, LSUHSC) who holds NIH R01 funding in tuberculosis pathogenesis and the influence of alcohol consumption on tuberculosis disease; Pamela Kozlowski PhD (Assoc. Prof. Medicine, LSUHSC) who is nationally recognized for her work in assay development for human mucosal antibody responses against HIV mucosal holds R01 funding in mucosal immunity against HIV infection; Angela Amedee PhD (Assoc. Prof. MIP, LSUHSC) had R01 funding in mother-to-infant lentiviral transmission in a macaques/SIV model and is currently funded through the NIH (P60) Center: *Alcohol, HIV infection and host defense*; Lucia Freytag PhD (Research Assoc. Prof. Microbiology and Immunology, Tulane HSC) has NIH funding to support her work in vaccine development and delivery; Joy Sturtevant PhD (Assoc. Prof. MIP, LSUHSC) who is R01 and R21-funded to study gene function in *Candida* and its interaction with host cells; Michael Hagensee MD PhD (Assoc. Prof. Medicine, LSUHSC) holds NIH and commercial funding to support clinical trials of a protein/adjuvant human papilloma virus vaccine and related immune assay development; Alison Quayle PhD (Assoc. Prof. MIP, LSUHSC) a reproductive immunologist studying interactions of host T cells with *Chlamydia* and with host mucosal epithelial cells, studies currently NIH-funded through the *Gulf South Sexually Transmitted Infections/Topical Microbicide Co-operative Research Center;* Jakob Reiser PhD (Assoc. Prof. Medicine, LSUHSC) holds R01 and P01 project funding for lentivirus, adenovirus and poxvirus and DNA-based vector development; Greg Bagby PhD (Prof. Physiology, LSUHSC) is Assoc. Director of the NIH-funded (P60) Center: *Alcohol, HIV infection and host defense*, and PI of the NIH (T32) training award: *Biomedical Alcohol Research Training Grant*; Paula Gregory PhD (Assoc. Prof. Genetics, LSUHSC) holds NIH R25 funding to support education and training of college students in biomedical research.

A high level of basic research interaction is already in place between individuals within this group of investigators, between intra-institutional departments, programs and centers, and also groups at different between institutions (as shown in diagrammatic form in the Figure below).



A variety of collaborative linkages have developed between individuals in a variety of departments and programs at LSUHSC, Tulane and Xavier and are ongoing (shown in red). Several of these interactions have given rise to five major new multi-disciplinary groups - four funded as NIH Centers or Programs and one Program formed utilising major funding from NIH and Dept. of Defense (makeup & linkages in green).

The multidisciplinary groups shown in the preceding Figure represent outstanding recent examples of highly productive interactions and leveraging of resources by Center members in a research area of particular local strength and include:

Bio-defense Vaccine Program Project (Clements, Freytag John, Grayson - Tulane) / Mandal -Xavier) is a collaboration among investigators with expertise in a range of disciplines. This program has won substantial funding from NIH and the Dept of Defense for anthrax and plague vaccine development

HIV-related Pulmonary Infections (Ramsay, Reiser – LSUHSC Gene Therapy Program / Shellito, Mason – LSUHSC Pulmonary) is concerned with new strategies to augment host defense against pulmonary infection with long-term emphasis on immune-deficient patients. This group has attracted major NIH Program funding to support this work (P01 HL076100).

Gulf South Sexually-Transmitted Infections/Topical Microbicide Cooperative Research Center (Martin, Hagensee - LSUHSC Infectious Diseases / Quayle - LSUHSC Microbiology & Immunology / Fidel – LSUHSC Center of Excellence for Oral & Craniofacial Biology / Pincus – LSUHSC Pediatrics & Research Institute for Children / non-Center Tulane investigators). This diverse group specializing in STD pathogenesis, reproductive immunology and population health has achieved national recognition and NIH Center Funding (U19 AI0619272).

Alcohol, HIV Infection and Host Defense (Nelson, Mason, Shellito – LSUHSC Pulmonary / Bagby – LSUHSC Physiology / Amedee - LSUHSC Microbiology & Immunology / non-Center Tulane Primate Center investigators). This diverse group won an NIH Comprehensive Center Grant (P60 AA09803) to study the impact and mechanisms of excessive alcohol consumption on host defense, with emphasis on HIV disease. Renewed in 2006 for a further five years in national competition.

COBRE Center of Excellence in Oral and Craniofacial Biology (Fidel – LSUHSC Dental / Luftig-LSUHSC MIP). This group won an NIH Center of Biomedical Research Excellence (P20 RR201160) for mentoring of translational researchers in oral infections, including *Candida*.

(2) CONTEXT FOR PROJECT

This group of local investigators enjoys an international reputation for excellence in research in viral, bacterial, parasitic, and fungal pathogenesis and many are at the forefront in developing methods to combat vaccine-preventable infectious diseases. Particular local strengths have been established in: (i) HIV pathogenesis and immunity and HIV-related pulmonary infections including tuberculosis and bacterial pneumonia, (ii) host-pathogen interactions in sexually-transmitted diseases (STDs), particularly *Chlamydia* and human papilloma virus, (iii) molecular pathogenesis and immunity in fungal infections, particularly *Candida*, (v) protective immunity against pathogens and toxins that represent potential bio-threats, (vi) development of delivery systems and adjuvants with broad vaccine potential, and (vii) effects of alcohol on disease pathogenesis, particularly HIV/AIDS & Tb.

In order to take the next step, a coordinated approach to the problems of vaccine development together with development of appropriate supporting infrastructure is now required. The *Center of Excellence for Vaccine Technology* will provide a critical focus for this important initiative. Working in a coordinated fashion, this group will foster robust translational research from bench to clinic and ultimately to the field. This group has unparalleled potential for vaccine development, including (i) cutting-edge research as outlined above, (ii) extensive experience in delivery systems for drugs and other biologically active molecules, (iii) a history of successful research interaction, (iv) extensive experience of leadership of successful multi-disciplinary and multi-institutional Centers and Programs, (v) a history of successful clinical trials, (vi) generation of related intellectual (see Appendix), and (vii) the strong support of local and state governing bodies.

Two additional factors are especially noteworthy. Firstly, these researchers have access to the nearby Tulane National Primate Research Center (Covington, LA) that includes a recently constructed large-scale primate BL3 facility. These facilities are of particular relevance for local HIV and tuberculosis vaccine research and development and will ultimately be important in Center vaccine projects against other infectious diseases. Secondly, there exists in Louisiana a unique patient base for vaccine-related clinical studies. Louisiana has among the highest rates of HIV, STD and Tb prevalence in the country. In the case of Tb, center researchers will have access to a large diagnostic and treatment clinic run by LSU Pulmonary/Critical Care faculty (Mason, Shellito, Nelson are key Vaccine Center personnel). In the case of *Chlamydia* and/or HIV, adolescents and young women are at particularly high risk of infection and rates are disproportionately high in low income groups, those not covered by health insurance and in ethnic minorities, each of which are highly represented in Louisiana. Young women infected with *Chlamydia* are at 3-5 times increased risk of acquiring HIV. The HIV Outpatients (HOP) Clinic and the New Orleans STD Clinic (within walking distance of LSUHSC and Tulane HSC) are staffed by LSU ID faculty, nurse practitioners, and clinic support staff.

(3) EXISTING SCIENTIFIC EXCELLENCE

The internationally recognized expertise of our group in molecular pathogenesis, host immunity, vaccine immunology, vector design and construction, and vaccine formulation and delivery provides an excellent foundation for the development of a real tower of scientific strength.

Members of our group collectively hold over $63M in total current research funding in these and directly related areas from the NIH ($57M including three Centers, one P01 Program, eleven R01s, six R21/s, one U01 co-operative agreement, and one N01 contract), the Department of Defense ($1.9M), and the State of Louisiana through the Board of Regents ($4.4M) and have a long history of publication in leading biomedical journals (as listed in biosketches). Our funding is complemented by an extensive history of executing multi-center clinical trials (Martin, Clements, Ramsay, Nelson, Hagensee, Kozlowski – collectively over 50 clinical trials performed since 1985) and in generating related intellectual property (25 patents, 1 start-up company: Clements, Cutler, Mandal, Ramsay, Luftig) [see: Appendix for summary of IP], solid evidence of our ability to develop basic research findings towards commercialization. The leadership team and other members of the group have extensive experience in successfully co-ordinating a variety of multi-institutional and multi-disciplinary research programs. A logical next step is to co-ordinate the expertise of this group to focus research efforts on specific issues concerned novel vaccines for infectious disease.

(4) MULTI-INSTITUTIONAL FOCUS

A feature of this proposal is the strong commitment of the three participating higher education institutions each based in New Orleans, as indicated in budgetary and supporting letters. Louisiana State University Health Sciences Center and Tulane University Health Sciences Center are, respectively, the largest public and private university biomedical research institutions in Louisiana. Each has an outstanding record of research achievement in infectious diseases and immunology. Xavier University of Louisiana is America's only historically black, Catholic university. Faculty achievements at Xavier, particularly in the sciences and in pharmacy, have earned national recognition. LSU Faculty based at the Research Institute for Children are renowned for their work on infectious diseases. Each of these participating institutions brings different and complementary strengths, and consequently, investigators will have access to a group of Core technologies / research strategies not currently available at any single institution.

Key features of the *Center of Excellence for Vaccine Technology* are (i) the participation of Faculty with demonstrated expertise in infectious diseases, immunology, and/or vaccine technology from all three institutions: LSUHSC (seventeen), Tulane (five) and Xavier (one); and (ii) the development of a series of strong, vaccine-related laboratory Cores located across each of the three institutions based on existing core structures and strengths and/or relevant scientific expertise within the group. Xavier will house the Vaccine Delivery/Nanotechnology Core, Tulane the Protein Core, and LSUHSC the Antigen Discovery, Vaccine Delivery/Vector, Immunology and Imaging Cores. The BSL-3 Lab core will be operated by LSUHSC Faculty based at the Research Institute for Children on the Children's Hospital campus. Thus, the combination, not only of investigators, but also of complementary resources located at each of the participating institutions, represents a true synergy that will provide an outstanding and necessary foundation for the Center.

As outlined in letters of support and budgetary documents, each of the Institutions has expressed strong support for the establishment and long-term development of the Center, comprising new matching funds for additional Faculty in vaccine research and commitment of new matching research space at LSUHSC, complementary funds for faculty hires in infectious disease and immunology at Tulane, and key Core equipment recently purchased at all three institutions, in addition to 10% faculty salary match and unrecovered indirect costs at each.

(b) Research Plan

(1) PROPOSED WORK

Overview: The key personnel participating in this application have demonstrated expertise in

microbial pathogenesis, host immunity, vaccine immunology, and vaccine design and delivery that provides an excellent foundation for the development of a real tower of scientific strength in the region. In particular, our work in: HIV pathogenesis and immunity and HIV-related pulmonary infections; STD's including Chlamydia and HPV; pathogenesis and immunity in fungal infections; protective immunity against bio-threats; delivery systems and adjuvants with broad vaccine potential; and the effects of alcohol on disease pathogenesis, has achieved international recognition and won substantial levels of external research funding for the State, with resultant beneficial downstream effects (including knowledge-based job creation, national recognition, and interest from biotech and pharma). What is urgently required to take this high level of achievement to the next step is co-ordination of these efforts towards the specific problems associated with vaccine development, together with the development of appropriate supporting infrastructure. A *Center of Excellence for Vaccine Technology* will provide this co-ordination and focus. The high degree of motivation of this group for the formation of this Center and for maximization of the benefits of P-KSFI establishment funding is reflected in the fact that key personnel have committed 10% effort to the Center but foregone budget requests for commensurate salary support provided as Institutional match.

Background: Infectious diseases are major and increasing threats to public health in Louisiana and worldwide. Each year, infectious diseases kill more than 17 million people, including 9 million children. In the United States, infectious diseases are now the third leading cause of death and impose an enormous financial burden on society. Vaccination is the most cost-effective means of controlling infectious disease morbidity and mortality.Unfortunately, we have now reached a new plateau in the war against infectious diseases. Although antibiotics and vaccines have been effective at reducing the morbidity and mortality of a variety of infectious diseases, new ones such as HIV/AIDS, Lyme disease, Hanta virus, and avian influenza virus are constantly emerging, while others such as malaria and tuberculosis have re-emerged in drug-resistant forms. We now also have to contend with the prospect of bio-terrorism and the potential devastating impact of intentional release of highly infectious microorganisms or bio-toxins on civilian populations. We also have an aging adult population with diminishing immune function, increased use of immunosuppressive agents for cancer, tissue transplantation, and autoimmune disease, and upwardly spiraling costs of health care delivery. In fact, the cost of administering vaccines by traditional vaccine strategies makes most current vaccines too expensive for use in developing countries.

Diseases such as HIV/AIDS and tuberculosis (whose re-emergence is also fueled by the HIV pandemic), occur at high rates in Louisiana, while the incidence of sexually transmitted diseases such as *Chlamydia* and gonococcus infection in the State is one of the highest in the nation. Together, these diseases, and AIDS-related bacterial and fungal infections, wreak untold human misery and cost billions of dollars in healthcare both in developed and in developing countries. Social factors such as alcohol and drug abuse are known to contribute to disease prevalence and can undermine the efficacy of treatments, and this likely includes vaccination. The development of novel approaches to vaccination for these conditions is now seen as **essential for their eventual control**, particularly in the face of the current dangerous explosion in microbial resistance to antibiotics and other drugs. Historically, vaccinology has been an empirical science and most successful vaccines were obtained by needle-based immunization with whole killed or attenuated organisms or inactivated toxins. That paradigm must now shift as the challenges before us become more daunting. Progress will depend on more detailed understanding of basic underlying processes of microbial pathogenesis, host immunity and immune evasion strategies used by the pathogens. Tailoring vaccines and therapies to individual pathogens based on this information and devising the most appropriate modes and routes of vaccine delivery will also be critical to their success.

The proposal:

(i) **Development of Essential Infrastructure – Cores**: This application seeks to establish a Center of Excellence for Vaccine Development in order to focus and co-ordinate the research activities of a world-class group of local investigators towards vaccine development. The by establishing appropriate infrastructure through the development of a series of Core Facilities designed to support different stages in vaccine research and development. Thus, our plan for Core growth and development is designed specifically to aid the flow of vaccine research from antigen discovery to vaccine preparation and delivery and through pre-clinical testing and analysis towards the development and conduct of clinical trials (and at any entry point in this continuum). In each case, these Cores will be developed through targeted enhancement of first-class facilities already in place across the three participating institutions, while each has a full-time manager hired on institutional or grants and is under the direction of Center PIs. Importantly, therefore, they will build significantly on major earlier financial commitments by LSUHSC, Tulane, Xavier, the State-funded Louisiana Gene Therapy Research Consortium, and funds from Center investigator's external grants, significantly adding to the value of these original investments. Strong institutional support for the use and development of these Cores for the Center of Excellence for Vaccine Development is expressed in letters and supporting documents that accompany this application.

We see the development of infrastructure dedicated for support of vaccine research in this way as an essential early step in the development of an effective vaccines center. These facilities will be and staffed and equipped for vaccine research primarily in Year 1 of the Center, and include Cores to support work at each different stages of vaccine research and development: All Center funds requested in Year 1 (apart from Administrative Core and Education Program budgets) are directed towards Core development (equipment, supplies and personnel). A significant proportion of the Center budget in Years 2-5 is for continued support of Core personnel and essential supplies. Maintenance of these Cores is critical for the success of the Center, indeed, the employment of Center-funded staff dedicated to vaccine Center-related activity within each Core will provide a direct entry point and an ongoing support pathway for Center investigators' research. Once Core vaccine research infrastructure is established it will immediately be available to support Center vaccine-related projects, as described below.

(ii) **Description of Center Cores**:

1). Antigen Discovery Core: the mission of the Antigen Discovery Core is to provide comprehensive genomics/proteomics/bioinformatics support to Center investigators through a considerable array of state-of-the-art analytical equipment and full data analysis capability. All components are located in adjacent space at LSUHSC, nearby the Imaging Core and include:

The Genomics Core was established by the LSUHSC Gene Therapy Program. Available equipment includes: Affymetrix microarray equipment for RNA profiling, a premier Rosetta bioinformatics package, an Illumina BeadStation® system for analyses of single nucleotide polymorphisms, and Illumina robot controls facilitating high volume automated sample processing. The Proteomics Core was established at LSUHSC through institutional and State funding. Available equipment includes: an ABI 4700 MALDI TOF-TOF mass spectrophotometer with Global Proteome Server Explorer workstation, a Perkin-Elmer Multi-Probe II sample handling workstation, a BioRad GS800 densitometer, a ProteomeWorks Spot Cutter with PDQuest software for gel imaging and spot picking, a BioRad 2-D gel electrophoresis system, an Amersham IPGPhor and Ettan 2-D gel electrophoresis system, a Typhoon imaging system and an Ettan handling workstation. LSUHSC has recently invested $800,000 to further enhance this facility through purchase of a nano- to micro-flow LC system for the MALDI (more efficient for biomarker discovery), an LTQ linear ion trap tandem mass spec (greatly increased sensitivity) and a 10-node parallel processor server and 5-node Bio-

works software to analyze data from the new LTQ mass spec (includes peptide sequencing/ database blasting for protein identification). Center researchers will have full access to this equipment.

To enhance the capacity for vaccine-related studies, upgrades of the Affymetrix scanner, of two Dual Core Xeon Workstations for Affymetrix users, of Rosetta Resolver bioinformatics software, of the Resolver server and workstations, and also an Illumina BeadXpress Reader will be purchased by the Center, also facilitating the use of protein and antibody arrays. Two research technicians dedicated to Center activities will facilitate Center investigator's research in the Antigen Discovery Core along with supporting basic laboratory supplies.

2). Protein Core: it is the mission of the Protein Core facility (PCF) to support and advance research capabilities at the Center by providing high quality protein and antigen purification support. The PFC is located at Tulane HSC and its major goal is to provide Center researchers with purified protein antigen for use in vaccine research. PCF personnel dedicated to vaccine-related Center projects (requested) will assist researchers in the selection of optimal recombinant expression systems (prokaryotic and eukaryotic), will sub-clone genes of interest into expression vectors, and will optimize protein expression and purification. The core will also assist in generating site-directed mutants of proteins of interest and in scaling up of recombinant protein production. Emphasis is placed on purity, with removal of bacterial endotoxin and contaminating immunogens from protein preparations, while minimizing degradation.

Available equipment includes: a New Brunswick Bioflow 3000 Fermentor with 3-liter and 10-liter vessels, a Microfluidizer cell disruptor and a Virtis lyophilizer, aBeckman DU-64 spectrophotometer, a flow cytometer, Beckman low- and ultra- speed centrifuges and rotors, autoclaves, gel dryers, a digital imaging system, a Dynatech Microfluor fluorescence plate reader, a Beckman liquid scintillation counter, a Beckman gamma counter, a Fuji phospho imager and software, and confocal and fluorescent microscopes. To facilitate vaccine-related Center research, a New Brunswick BioBlo 4500 20-liter bioreactor for large scale purification and a BioRad BioLogic Douflow chromatography system will be purchased with Center funds, along with supplies. Two research technicians dedicated to Center research and development projects will be employed by the Center.

3). Vaccine Delivery Core

The Vaccine Delivery Core has two major components: a nanotechnology division and a vector technology division. Both are first-class core structures previously established through LSUHSC and Xavier funding, and through investment by the State-funded Gene Therapy Consortium.

(a) **Nanotechnology**: it is the mission of the Vaccine Delivery/Nanotechnology Core facility to support and advance vaccine research capacity at the Center by providing novel and innovative vaccine delivery formulations. The major goal of the Core, located at Xavier University, is to maintain a state-of-the-art innovative polymeric vaccine delivery research facility in order to support inter-disciplinary research. Core personnel will provide leadership in planning, designing, and implementing innovative nanotechnology and will also assist investigators in conducting pre-formulation and formulation studies of any potential novel vaccine delivery system for preclinical and NDA studies (New Drug Application following USFDA guidelines). Nano-delivery technology will be developed and/or adapted, in collaboration with Center researchers, to address the special requirements of either systemic or mucosal (ie. intranasal, pulmonary, oral, or intra-vaginal) particle-mediated delivery of peptides, proteins and/or recombinant DNA vaccines in preclinical and, ultimately, clinical studies. Targeted particle- or lipid-mediated delivery either of proteins via novel routes (eg. transcutaneous) or of alternative recombinant vaccine vectors is already under development in the Core in NIH and/or DOD-funded studies (in collaboration with John Clements and Alistair Ramsay, respectively) and will also be made available to other Center investigators.

Currently, our state-of-the-art NIH-funded nanotechnology research laboratory is equipped with R&D-scale pharmaceutical formulation equipment, with research staff that have developed unique skills in micro-encapsulation for controlled release. Available equipment includes: a Scanning Electron Microscope, a Fluid Bed Coating machine, a Super Critical Fluid (SCF) particle preparation equipment, a high pressure homogenizer to prepare lipid nanoparticles, a particle size analyzer, a zeta sizer, and an automated dissolution apparatus. Other Core equipment includes a preparatory ultracentrifuge, an analytical ultracentrifuge, a fluorescence spectrometer, and a fluorescent microscope. Specifically for vaccine-related research, a Laboratory Mini Spray Drier (forms nanoparticles ideal for pulmonary vaccine delivery) and a Waters Acquity SQD LC/MS System (facilitates measurement of small quantities of vaccine material and their preparation for delivery) will be purchased by the Center. A Core research technician will be dedicated for Center projects.

(b) **Vectors:** The Vaccine Technology/Vector Core will greatly facilitate co-operative Center vaccine research through the design, engineering, preparation and purification of new recombinant vaccine vectors and novel vector technology. The Vector Core is based at LSUHSC . Core services include construction of new recombinant vectors, and large-scale preparation of recombinant vectors for use, quality control. The Core maintains an extensive inventory of plasmids and cell lines that are useful in the development of recombinant vectors. Several vaccine vector systems are currently available through the Core: DNA vaccines, replication-defective adenoviral vectors, lentiviral vectors, and vectors based on oncogenic retroviruses including mouse stem cells virus (MSCV). More recent additions include vectors based on poxviruses (vaccinia or fowlpox) and adeno-associated virus (AAV). Current serotypes of the latter include AAV1, 2, 5, 7, 8, 9 and AAVrh10.

The Core is already equipped for manufacture of the above mentioned vector systems through LSUHSC, Gene Therapy Consortium and NIH funding. To facilitate vaccine Center studies, a research technician will be dedicated to vector development for Center researchers along with supporting supplies. An electroporator device for in vivo inoculation of engineered DNA vaccines will be purchased to support Center pre-clinical vaccine studies.

4). BSL-3 Core: The BSL-3 Core is dedicated entirely vaccine-related studies of Center investigators. This includes pathogens of current research interest to Center researchers (eg. M. tuberculosis) and any future studies in disease models not involving select agents (eg, SARS). The Core is located at the Research Institute for Children (RIC). Key features include two independent laboratories, procedure and equipment rooms, and a work room. The facility has been established for BSL-3 operation and includes laminar flow hoods and other basic equipment. Specialized equipment for work with *M. tuberculosis*, including a Glas-Col Inhalation Exposure system, and waste dumpster, has been purchased through funding from the Center Director's NIH grants (Ramsay project, P01 HL076100). Basic operating equipment for dedicated vaccine Center studies will be purchased including: cell and bacterial incubators, centrifuges, microcentrifuges, a convected shaker, microscopes and a computer, along with two HEPA-filtered animal racks with cages. A facility manager dedicated to Center operations will assist investigators with growth of microorganisms, inoculations and sampling of experimental animals and their care.

5). Immunology Core: the Immunology Core will serve Center Investigators in the measurement of immune responses in vaccine-related studies and data analysis. The Core is located at LSUHSC in space near all other LSU-based core facilities described in this proposal. State-of–the–art equipment includes: a FACSAria (9-parameter analysis/sorting), a FACS LSRII (18-parameter analysis), a FACS Calibur (4-parameter analysis), a Gel Logic Imaging system for Western Blot, a Dynatech ELISA system and reader, a BioRad multiplex system for multiple parameter (cytokine/chemokine) analysis in small fluid samples, a Tri-Carb Liquid Scintillation counter, a Perkin-Elmer Top Count Gamma Scintillation counter, and an Olympus Fluorescence Microscope

station. An AID ELISPOT Reader and workstation will be purchased, while a research technician will be dedicated to support Center vaccine research and perform data analysis. The Core has a fully qualified FACS operator (Constance Porretta MS). Full FACS acquisition and data analysis will be provided to Center researchers. A sub-component of the Immunology Core available to Center investigators will be developed by Pam Kozlowski PhD (LSUHSC, key personnel in this application) in order to facilitate the measurement of antigen-specific mucosal IgA antibodies in non-human primate vaccines and in human samples from clinical vaccine trials, particularly for HIV and STDs. Her lab at LSUHSC is fully equipped for this role and will also function as a core for measurement of macaque mucosal antibodies in an NIH-funded P01 (AI71306, Johnson, PI, New England Regional Primate Research Center/Harvard). James Robinson (Tulane) and Seth Pincus (LSU/RIC) are also a major resource for validated human and monkey HIV/SIV neutralizing antibody study and measurement within the Center.

6). Imaging Core: the mission of the Imaging Core is to provide histology, microcopy, imaging services to support and advance vaccine research in the Center. The Core is located in LSUHSC Gene Therapy Program space adjacent to the Antigen Discovery Core (Genomics/Proteomics) and offers expert advice on experimental design and interpretation of results. Available equipment includes: a Leica CM3050S Cryostat with CryoJane Module, a Shandon Hypercenter XP Paraffin Processor, a Shandon Histocentre Paraffin Embedding Station, a Shandon Finesse ParaffinMicrotome, a Leica DMRXA deconvolution microscopy system, a BioRad Radiance 2100 Laser Scanning Confocal System, a Nikon E600 brightfield / epifluorescence microscopy system, and a Leica Mz75 brightfield / epifluorescence stereomicroscopy system. The Core has also recently purchased a state-of-the-art P.A.L.M. Laser Microdissection System capable of dissecting minute fragments of target tissue from sections for detailed histological, immunological or genomic analyses. This offers significant advantages to Center researchers for gene-based and phenotypic analyses of minute regions of tissue (as few as 10 cells) in studies of host/pathogen interaction.

Core development for vaccine-related projects within this Center will include the purchase a Xenogen IVIS® SPECTRUM imaging system, allowing real-time imaging to non-invasively monitor and record cellular and genetic activity within a living organism with attendant advantages of high throughput (of mice) and high-sensitivity in vivo imaging of both fluorescence and bioluminescence with resolution (at 3.9cm field) to 20 microns. This equipment will facilitate in vivo localization and tracking of labeled cellular and genetic material within an organism in studies of disease pathogenesis and host response. A research technician dedicated to Center activities will be placed in the Core along with supporting supplies.

7). Regulatory and Clinical Trial Support Core: given the extensive regulatory hurdles that need to be overcome before clinical testing can begin and the degree of documentation that is necessary it is important that Center investigators are well-versed in these requirements and have resources at their disposal to help address these issues. Beginning in Year 2, a support core will be established that serves both educational and practical roles at all levels of Center activity. Thus, the Core will offer instruction and practical assistance in clinical trial development, in the preparation of IND and other relevant documentation for the FDA, in intellectual property and patent filing, and ultimately in research commercialization. The initial scope of this Core (one employee with regulatory expertise) will be expanded as required at the discretion of the Steering Committee.

8). Additional Core Support and Other Resources: Center researchers will also have access to the Biostatistics Core (led by Donald Mercante PhD) within the LSUHSC Center of Excellence in Oral and Cranofacial Biology (Paul Fidel PhD, Director, and key personnel on this application).

Additional key facilities for vaccine research and development that are available locally for funded Center projects include: (i) the Tulane National Primate Research Center (with BSL-2 and BSL-3 facilities for vaccine-related studies in non-human primates, primarily rhesus macaques); (ii) the Tulane/LSU General Clinical Research Center (NIH M01RR05096 – currently funded through 11/07 to support local clinical trials with inpatient beds, outpatient facilities, nursing support and laboratory services); and (iii) the HIV Outpatients (HOP) Clinic and New Orleans STD Clinic staffed by LSUHSC ID faculty and with clinical laboratory facilities (as described in (a) (2)) that provide an outstanding resource for clinical studies including vaccine trials for HIV and STDs.

(iii) Project Development: Once the above mentioned Core infrastructure is established it will be immediately available to support vaccine-related studies for Center investigators, whether funded through PI's external grants or through the Center. Availability of Center funding for such projects will be determined by the Center Steering Committee (CSC) that will meet bi-monthly, or as required (see Management Plan below) to advise the Director, among other matters, on research and development priorities for the Center, including funding for vaccine research projects. A proportion of the requested Center budget in Years 2-5 has been set aside to support scientifically meritorious new vaccine projects, with priority given to those that: (a) are multi-disciplinary and collaborative, in that they draw on complementary expertise within the Center to address key priority areas, and/or (b) are novel, in that they have potential for the generation of new intellectual property; and/or (c) have the potential to progress through pre-clinical studies to clinical trials.

Initial Center vaccine projects will reflect new interactions across current areas of expertise. Initially, strong, interdisciplinary teams will be organized around several themes arising from current expertise, defined as follows: (i) HIV pathogenesis and immunity &HIV-related pulmonary disease, including Tb, pneumocystis [Ramsay, Amedee, Bagby, Luftig, Mandal, Mason, Nelson, Pincus, Robinson, Shellito], (ii) sexually-transmitted diseases, particularly Chlamydia and HPV [Martin, Hagensee, Quayle], (iii) fungal infections, particularly Candida [Cutler, Fidel, Pincus, Sturtevant], (iv) protective immunity against potential bio-threats [Clements, Freytag, Pincus, Ramsay], and (v) delivery systems and adjuvants with broad vaccine potential [Mandal, Clements, Grayson, John, Reiser]. There will be significant overlap and interaction between these teams as projects progress.

Summaries of four new projects that exemplify the potential for multi-institutional and multidisciplinary interactions to push existing research towards novel approaches for vaccine development are outlined below. In addition, It is important to note that our early discussions concerning the formation of this Center (with its attendant benefits for local vaccine research) played a critical role in the building of underlying concepts. Each project was developed to take advantage of the broad scope offered by new collaborations and the proposed development of dedicated vaccine Cores and, importantly, several have the capacity to advance to clinical trials within the funding period. Most were conceived either at, or in discussions subsequent to, preliminary vaccine Center planning meetings held in the summer of 2005 (immediately prior to Katrina and its prolonged and disastrous aftermath) or, more recently, in February 2007.

Project 1: HIV vaccines against gp41env

Primary Investigators: Kozlowski and Ramsay (LSUHSC)
Center Collaborators: Robinson, Clements (Tulane), Pincus (LSUHSC/RIC), Mandal (Xavier)
Center Cores: Protein Purification, Vaccine Delivery, Immunology
Other facilities: Tulane Primate Center, HIV Outpatients Clinc

Rationale: There is a lack of HIV vaccine candidates capable of generating strong antibody responses in human vaccine recipients. In addition, current HIV vaccines are not generally designed for administration at mucosal surfaces, the most effective way to induce immune

responses in the intestinal and genital tract mucosa, which serve as major reservoirs of HIV. In this regard, an HIV vaccine that generates secretory IgA against the gp41 envelope protein could be highly effective for preventing mucosal HIV transmission. The highly conserved gp41 protein binds to b-galactosylceramide (GalCer), a sphingolipid ubiquitously expressed on epithelial cells. Binding has been shown to result in transport of infectious HIV particles across intestinal epithelial cells in vitro. The 2F5 anti-gp41 monoclonal antibody blocks this transport by binding to a sequence known as ELDKWA within the gp41 GalCer binding site. Interestingly, 2F5 also mediates broad, cross-clade neutralization of HIV in conventional neutralizing assays.

Outline: We propose, with the aid of the Vaccine Delivery and Protein Cores, to develop a gp41 DNA/protein-based vaccine formulation that induces broadly neutralizing as well as "entry blocking" antibodies. In mice, nasal immunization with DNA can be used quite successfully to generate antibodies in both blood and secretions. Therefore, we will first identify an optimal gp41 DNA sequence by analyzing the function of gp41 antibodies induced in mice nasally immunized with DNA plasmids engineered to express the 36 aa gp41 GalCer binding site vs slightly longer sequences (Immunology Core). (Year 1). In humans, DNA vaccines are less effective than in mice for induction of antibodies, therefore, the "Ab-inducing" component of our vaccine formulation will consist of a truncated recombinant gp41 protein delivered in liposomes/ nanoparticles developed in conjunction with the Vaccine Delivery/ Nanoparticle Core. Our approach to the design of this protein is unique. With the exception of the GalCer binding site, we will omit the gp41 extracellular domain. Also in contrast to others, we will express the entire gp41 transmembrane domain and a portion of the cytoplasmic tail. This should facilitate the incorporation of gp41 into liposomes in such a manner that the gp41 more closely mimics native conformation. In Year 2, we will test the immunogenicity of our gp41-liposomes/nanoparticles using a DNA prime/protein boost regimen in mice. The vaginal route will be included in these studies as local vaginal immunization in women is more likely to generate greatest HIV-specific IgA antibody levels in cervicovaginal fluids when compared to other routes **[1]**. Standard neutralizing antibody preparations for reference use in antibody assays will be obtained within the Vaccine Center from the laboratories of James Robinson and Seth Pincus. In Year 3, non-human primate studies with GLP-grade material would be performed at Tulane Primate Center to evaluate protective efficacy, with testing facilitated by the Immunology Core. If successful, this study will to move towards local clinical trials in Yrs 4 and 5.

Project 2: Antigen discovery for protective vaccination against Burkholderia mallei, a potential bio-threat

Primary Investigators: Clements & Freytag (Tulane)
Center Collaborators: Mandal (Xavier), John & Grayson (Tulane), Ramsay (LSUHSC)
Center Cores: Antigen Discovery, Protein Purification, Vaccine Delivery, Immunology

Rationale: The post-September 11 release of anthrax spores resulted in five civilian deaths, seventeen infections, and required that more than 30,000 individuals undergo prophylactic antibiotic therapy. This event also highlighted the need for improved vaccines that would be appropriate for pre- or post-exposure immunization of civilian and military populations against anthrax and other potential bioterrorism agents, including Burkholderia mallei, the causative agent of glanders in horses. B mallei is highly infectious for humans by the aerosol route and is capable of producing rapid onset pneumonia, bacteremia, necrosis of the tracheobronchial tree, and pustular skin lesions, leading to death in 7-10d without appropriate antibiotic treatment. There is no effective vaccine, although a number of killed-cell and live-attenuated candidates have been evaluated in animal models.

Outline: The approach taken here exemplifies an integrated Core-based strategy that is applicable to the development of new approaches for vaccine development against a variety of pathogens. Such an approach is greatly facilitated within the scope that is offered by a multi-disciplinary Center. In

Years 1 and 2, we will identify potential vaccine targets in B. mallei in the Antigen Discovery Core by genome sequence analysis and proteomics, using a combination of techniques that have been shown to be successful for identifying vaccine candidates in other Gram-negative bacterial pathogens. We will examine the genome sequence of B. mallei for open reading frames (ORFs) that potentially encode novel surface-associated proteins. Reverse vaccinology will be used to separate and identify immunoreactive outer membrane proteins. This technique makes use of 2D/PAGE, Western blot analysis and mass spectrometry to identify the potentially encoded ORFs. These putative ORFs will be cloned, sequenced, and expressed in E. coli as His-tagged fusion proteins and purified by affinity chromatography. Successfully expressed and purified proteins will be used to immunize mice, whose sera and bronchioalveolar lavage fluids will be evaluated for reactivity against whole, intact B. mallei by enzyme-linked immunosorbent assays and fluorescence-activated cell sorting (Years 2-3). Importantly, we will utilize immunization techniques, including novel adjuvants and delivery systems, that we have shown to induce protection against other aerosolized bio-threat agents (e.g. Yersinia pestis). The open reading frames giving rise to immunogenic and/or immuno-protective proteins in the preceding experiments will also be engineered into recombinant vaccine vectors developed specifically for systemic or mucosal delivery (Vaccine Delivery/Vector Core). These will be tested in prime-boost combination (ie. vector-vector or vector-protein) for their capacity to elicit heightened and/or sustained protective immune responses (Years 2-3). The strength of this approach is that it ties together genomic/proteomic approaches to antigen identification with the vaccine technology necessary to induce and evaluate an appropriate immune response against aerosolized bio-threat agents. Through this initiative, we should be able to identify potential vaccine candidates for prevention of aerosolized B. mallei and map out strategies for developing vaccines against other pathogenic bacteria, including bio-threat agents, than can rapidly be progressed towards tests for protective efficacy in pre-clinical studies in appropriate containment facilities.

Project 3: Second generation local vaccines against human papillomavirus (HPV)

Primary Investigators: Hagensee (LSUHSC)
Center Collaborators: Kozlowski (LSUHSC), John & Grayson (Tulane), Mandal (Xavier)
Center Cores: Protein Purification, Vaccine Delivery, Immunology

Rationale: Infection with human papillomavirus (HPV) has been implicated in the etiology of the majority of ano-genital malignancies, accounting specifically for more than 90% of cervical carcinomas. Recently, prophylactic vaccines that prevent infection with HPV have been developed against HPV types 16 and 18 that historically have caused about 67% of cervical cancers and dysplasias **[2,3]**. These vaccines generate potent type-specific humoral antibody responses that appear to be almost 100% effective in preventing type-specific infection and HPV-related disease against the types contained in the vaccine. However, there appears to be little or no cross-protection against other HPV types. It has been clearly demonstrated that the HPV types contained in cervical cancer tissue varies greatly geographically. Indeed, we found that the most prevalent high-oncogenic risk HPV types in a HIV-negative gynecological referral clinic in New Orleans, LA were 16, 52, 58, 35, 51 & 18 in rank order **[4]**. Types 16, 31, 51, and 52 accounted for 30% of low-grade dysplasia cases and 45% of high-risk dysplasia. Similar geographical differences elsewhere could account for the lower than expected overall protection rate found with the new HPV vaccines (40-45% reduction in cervical dysplasia compared to the expected 67% mentioned above despite near 100% vaccine seroconversion). The goal is to develop second generation HPV vaccines applicable to the population of Louisiana and to similar populations elsewhere in the United States. Our approach also explores novel mucosal vaccination approaches that have been shown to be more effective for induction of local antibody than traditional intramuscular delivery routes.

Outline: In Years 1 and 2 we will continue to generate preliminary data that will inform the development of our study. Cervical swabs from women attending referral gynecological clinics in New Orleans, Baton Rouge, Shreveport and Lafayette (n=200 at each site) will be tested for HPV by utilizing the Roche reverse line blot assay. In addition, cervical dysplasia biopsy and cancer tissue from these 4 referral areas will also be tested for the presence and types of HPV present. During this time we will also focus on production systems for HPV virus-like particles (VLP) comparing E. coli, yeast, baculovirus, and vaccinia virus expression systems each previously shown to be effective for generation of high quantity HPV VLP. The most effective and efficient expression system will be scaled up towards GMP production. We will focus on prevalent 'Louisiana' virus types identified in our clinical studies. Meanwhile, using existing VLP, we will focus on the development of safe and improved mucosal delivery systems including (i) effective nanoparticle-based strategies for vaginal delivery in collaboration with Tarun Mandal and the Vaccine Delivery/Nanoparticle Core, and (ii) vaginal tampon-based delivery in collaboration shown by Pam Kozlowski to be effective for induction of local antibody production against a several vaccine antigens (unpublished). Systemic (IM) and mucosal delivery strategies will also be tested in prime-boost combination. These approaches will be tested for safety and immunogenicity (serum and vaginal antibody levels) in mice, rabbits and macaques in Years 2-4 of the project (Immunology Core). Beginning in Year 4 of the project, the safety and immunogenicity of a second-generation mucosal HPV vaccine based on local HPV types, and on the optimal delivery approaches determined in pre-clinical studies, will be appropriately tested against the current commercially available vaccine in clinical trials using local female volunteers and clinical support infrastructure developed within the Center (see Center cores).

Project 4: Vaccines for *Chlamydia* targeting antigens associated with persistence

Primary Investigators: Quayle & Martin (LSUHSC)
Center Collaborators:Kozlowski,Ramsay (LSUHSC), Mandal (Xavier), Clements, John (Tulane).
Center Cores:Antigen Discovery, Protein Purification,Vaccine Delivery, Immunology

Rationale: Annual US healthcare costs for treatment of Chlamydia infection exceed $2.4B **[5]**. Louisiana is among those States with the highest *C. trachomatis* prevalence rates. The organism is an obligate intracellular bacterium with tropism for columnar epithelial cells, and the most common site of infection is the endocervix. Similar to many of the other STD's, there is no *C. trachomatis* vaccine and, to date, insufficient detailed knowledge of host-pathogen interactions that would inform the development of effective vaccine strategies. *C. trachomatis* may enter a persistent state *in vivo* in humans resulting in chronic low grade infections in some women **[6].** Recent data suggest that the Chlamydiae have evolved to control the transition between acute and persistent growth. Thus, in contrast to the classical paradigm of persistence as a general stress response, these new findings suggest the possibility that persistence may be a regular occurrence in the life cycle and is used by the organism to evade the host immune response. Our patient data support the *in vitro* evidence that persistence consistently appears be a normal part of the chlamydial life cycle *in vivo*. We have recently developed an *in vitro* model using polarized primary endocervical epithelial cells. As opposed to the continuous cell lines that are used to isolate *C. trachomatis,* when these cells are infected with recent clinical human isolates it appears that the persistent form of the infection is spontaneously established. The possibility that persistence is a 'routine and rapid' strategy used by *chlamydia in vivo* has important implications for vaccination.

Outline: We propose a stepwise series of studies that take a unique approach to the due process needed to develop a vaccine for *Chlamydia*. Based on our recent findings in *Chlamydia*-host interactions, we believe that identification of antigens exposed during persistent infection may be critical. The specific expertise of the Quayle / Martin Laboratories in *Chlamydia*-host interactions, the world-class local clinical and diagnostic laboratories, and the unique local patient base all place us in a

unique position to undertake this work. The expertise and multi-disciplinary focus provided by the Vaccine Center will be essential for us to develop these novel concepts towards vaccine development. Initially, using the facilities and guidance provided by the Antigen Discovery Core, we will undertake microarray and Real-time PCR studies and proteomics to identify and compare chlamydial antigens that are highly expressed in acute and in persistently infected women, and also in polarized, persistently-infected human primary endocervical epithelial cells (Years 1-3). This novel approach should reveal proteins that can be further characterized and tested for potential inclusion in a vaccine. We will also use commercial Pentamer/Ultimer technology to probe T cell responses in longitudinal samples from specific cohorts of patients in order to directly identify and then further characterize a variety of immunogenic peptide T cell targets in *Chlamydia* after their selection by the host immune system over time (Years 1-3, Antigen Discovery Core, Immunology Core). Together, these approaches will help to delineate a variety of potentially immunogenic antigens that may be cloned, or their gene sequences synthesized, for inclusion in new vaccination strategies appropriate for T cell induction, particularly vector-based approaches and nanoparticle delivery (Vaccine Delivery Core). Protein or shorter peptide sequences would also be generated for tests of their immunogenicity and protective efficacy in preclinical infection models following systemic or mucosal delivery of different vaccine formulations (Years 2-4, Immunology Core, Imaging Core). Ultimately, the potential significance of this work for *Chlamydia* vaccine development will be clear only through design and testing of these strategies in humans. Development of effective vaccine delivery systems (possibly with compatible adjuvants) has been particularly challenging for mucosal pathogens, and this is especially so in the reproductive tract - a uniquely-regulated site with regard to immunity. However, the broad expertise in mucosal pathogens and delivery systems that is available locally through the Center (Kozlowski, Ramsay, Clements, Mandal, Vaccine Delivery Core) offers possibly unique scope for a powerful collaborative approach to this problem. Importantly, the resultant vaccine technology would be broadly applicable to many STD's.

In year 2, the Center will also facilitate the development of a further series of novel multi-disciplinary vaccine-related projects based on particular foci of group expertise and that have strong potential to rapidly inform new vaccine development, including:

Development of a multi-clade HIV vaccine based on patented (LSUHSC) virus-like particles

Primary Investigators: Ron Luftig and Pam Kozlowski (LSUHSC)
Center Collaborators: James Robinson, John Clements (Tulane), Seth Pincus (LSU/RIC)
Center Cores: Protein Purification, Vaccine Delivery, Immunology, Imaging

Development of synthetic vaccines for Candida albicans - targeting novel cell wall antigens

Primary Investigators: Cutler, Pincus LSUHSC/RIC, Sturtevant, Fidel (LSUHSC)
Center Collaborators: Mandal (Xavier), Clements, Freytag (Tulane)
Center Cores: Protein Purification, Vaccine Delivery, Immunology, Imaging

Targeting proteins expressed by *M. tuberculosis* during the development of non-replicating persistence in the host using novel vector-based approaches

Primary Investigators: Ramsay, Mason (LSUHSC)
Center Collaborators: Mandal (Xavier), John, Grayson (Tulane), Shellito (LSUHSC)
Center Cores: Protein Purification, Vaccine Delivery, BSL-3, Immunology, Imaging

The effects of alcohol consumption on prime-boost vaccination strategies against SIV and *M. tuberculosis* in rodent and non-human primate disease models

Primary Investigators: Nelson, Bagby, Mason, Ramsay (LSUHSC)
Center Collaborators: Mandal (Xavier), Shellito (LSUHSC)
Center Cores: Vaccine Delivery, BSL-3, Immunology

(iv) Research Organization, Education and Training: An important component of Center activities will be training of students and postdoctoral scientists and mentoring of junior faculty. Students and postdoctoral trainees will be attached to at least two participating Center laboratories to facilitate their exposure to a variety of ideas and approaches to vaccine research and to assist with the development of interdisciplinary projects between different labs. Junior faculty who join the Center will each have two mentors from among participating Center PI's.

The Center will also develop a summer internship program, whereby interested students from local high schools will gain early exposure to Center vaccine research activities. This will build on the outstanding success of an existing program in Gene Therapy (Alistair Ramsay, Director) that, in 3 short years, has provided research experiences for 50 of Louisiana's best and brightest High School and undergraduate students. This was developed by Paula Gregory PhD (NIH-funded for science education) and will form the basis of her vaccine Center program. Students will learn about cutting edge vaccine-related research at regular weekly seminars, and also Responsible Conduct of Research, how to write an abstract and how to put together and present a Poster. Many of the students in the Gene Therapy summer program have subsequently attended national scientific meetings and co-authored publications with their mentors. 20% of participants are under-represented minorities and over 50% are women. One participant is now enrolled in the PhD training program at LSUHSC.

The Center will support two components: (i) summer research internships for five students (to participate in an intensive 10-week research project in vaccine Center labs) and (ii) two Research Scholar positions that will support students while they conduct a year-long research project that is an expansion of their summer project. Both groups will attend Vaccine Center seminars, lab meetings and will receive instruction on effective scientific writing, presentations and guidance concerning career opportunities in this research area. Research Scholars will continue their regular coursework while also receiving credits for their time in the lab. The summer program has formative evaluations that are used to fine-tune the program and summative evaluations. Tracking the career paths of participants will provide a measure of the long-term impact of the program on career choices..

A seminar program will alternate with a journal club every 2 weeks. Each session will feature a single presentation of 45-60 minutes by distinguished visiting speakers or senior/junior faculty, or two 30-minute presentations by students and postdoctoral trainees. Students, postdocs and junior faculty will be expected to present a seminar and a journal club each year. Additionally, junior faculty will be expected to present, to an audience of their peers and senior faculty, an annual "work-in-progress" session, including their projects, any grants or manuscripts in preparation, and any other topics deemed relevant by the group. Presentations will be formally evaluated and discussed with mentees. Career development plans for junior faculty will also be devised with approval of relevant Departmental chairs. An annual Center Symposium will comprise scientific presentations, with time for discussion, planning and informal interactions. Educational workshops will be held with our Regulatory and Clinical Trial Support Core staff (assisted by outside speakers as required), offering instruction in clinical trial development, preparation of IND and other relevant documents for the FDA, intellectual property and patent filing, and commercialization.

(2) PROJECT IMPACT

It is noteworthy that the research field of immunology and infectious disease has recently been identified as a priority area for financial support in the State for its potential to aid economic recovery in Louisiana post-Katrina. It is also due, in large part, to the expertise of local researchers, many of whom are involved in the current application. These two factors are inextricably related. The

development of this Center will help to promote LSUHSC, Tulane HSC and Xavier as leading Institutions in vaccine development and delivery technology, key areas with enormous potential for development of new IP, biotechnology and investment by major pharmaceutical companies. However, this potential will only be realized if the appropriate initial seeding investment is made.

We recognize the need for a significant return for this P-KSFI investment for Louisiana. This group has an outstanding record of leveraging State investments to bring in large amounts of Federal and Industrial funding, and has also been highly successful in creating new intellectual property and commercialization in their diverse research and development activities in infectious disease and immunology (24 patents awarded, 5 patents pending; 1 start-up company created, see Appendix). The consolidation of their efforts in the area of vaccine research that will be provided by this Center will elevate these achievements to a higher level, providing greater opportunities for enhanced leveraging of funds in a high priority area that profoundly impacts health care. Given the pressing need for novel vaccine strategies for infectious disease and the public and commercial priority currently afforded high quality research in this area, we would expect to leverage P-KSFI funding by the end of the 5-year period of the award at least in a ratio of 1:1 with new Federal and Private Foundation awards and Industrial funding. This will generate new IP leading to further patents and commercial licensing opportunities.

In the post-Katrina phase, there is increased local and State support for an energized biotechnology sector in New Orleans that will significantly boost local economic recovery and development. The establishment of this Center would create a vital component of such an enterprise in an area of R&D that has huge potential for economic growth. It will also help to retain important intellectual capital in the State in what can only be described as difficult times. A co-ordinated push in this area is well overdue considering the high level of local expertise that currently exists in this field of endeavor. In providing such a cornerstone for consolidation of vaccine R&D in the South, and by helping to leverage other local and State-funded developments that are already underway (such as the Louisiana Cancer and Gene Therapy Research Consortia and the New Orleans BioInnovation Center) the Center of Excellence in Vaccine Development will have a key role to play in local development of the biotechnology industry and in attracting major pharmaceutical investment to the region.

An obvious spinoff of these developments will be the creation of a highly significant number of new knowledge-based jobs in the State. As mentioned earlier in this application, this group of researchers currently holds nearly $59M in Federal grant funding won for the State and a significant portion of this supports the creation of a large number of technical and other scientific support positions primarily for young citizens of the State, many of whom have recently graduated from local and regional institutions of higher education.

Of immediate benefit, P-KSFI funding for the Center of Excellence for Vaccine Development will also contribute significantly to the post-Katrina recovery of research and education infrastructure in Louisiana. A successful Center will certainly attract outstanding new Faculty, postdoctoral fellows and graduate students into the region. We have also outlined a compelling education and training component in our research plan to stimulate and develop this process from within the Center and the State by capturing the interest of the best and brightest local students as early as High School and exposing them to the full range of scientific endeavor in this exciting area of research and development. The combined effects of stimulating the interest of scientists and potential trainees both externally and from within the State will have positive spin-off effects for each of our Institutions and other regional research enterprises.

Finally, Louisiana is in the invidious position of having some of the highest rates of chronic infectious disease in the nation. Among other conditions, Chlamydia infection, HIV/AIDS and Tb all occur at high incidence and ultimately cost millions of dollars in health care. With its goal of

developing vaccines against these and other diseases, the Center will be in a strong position to positively and significantly impact public health both in the region and beyond.

(3) MANAGEMENT PLAN

The Center *Administrative Core* will be based at LSUHSC and will comprise a business manager (50% effort) and an administrative assistant (100% effort), who will assist the Director. A *Center Steering Committee* (CSC), comprising the Director and four senior investigators (including at least one from each partner institution) and initially including John Clements PhD (Tulane HSC), Seth Pincus MD (LSUHSC/RCI) and Tarun Mandal PhD (Xavier University), will meet bi-monthly, or as required, and will be charged with responsibility for the organization and conduct of scientific, clinical and administrative matters pertaining to the Center, including IP agreements. It should be noted that this group has already met on two occasions to discuss the formation and structure of the Center and played a significant role in the preparation of this proposal.

An *Internal Advisory Board* (IAB) comprising the Vice-Chancellor of Academic Affairs or equivalent at each partner institution (or their nominee), chaired initially by Dr. Joseph Moerschbaecher (Vice-Chancellor for Academic Affairs and Dean of the Graduate school at LSUHSC and an R01-funded investigator), will meet 6-monthly and will advise the Director and CSC on the progress of the Center.

An *External Advisory Board* (EAB) of 3 members comprising experts in basic vaccine/therapeutics research, translational/clinical research, and biotechnology/ commercialization will review the progress of the Center and give advice on future directions to the CEC yearly. The EAB will be chaired by Professor Lawrence R. Stanberry MD, PhD (John Sealy Distinguished Chair and Professor of Pediatrics, Director of Sealy Center for Vaccine Development, University of Texas Medical Branch and Children's Hospital, Galveston TX; see attached letter of support). Additional EAB membership will be determined by the CSC with the approval of the IAB.
anthrax and plague

(4) PERFORMANCE MEASURES AND OBJECTIVES

By end of Year 1our goal is to have each of the Cores fully functional for Center projects, including all new equipment, and research assistants in place and fully trained in usage of equipment and data collection and analysis. Interdisciplinary project teams (as delineated in the Research Plan – Project Development) will have formed and met at least 3 times and the 4 initial projects listed in Research Plan are underway. Seminar and journal club programs underway, annual retreat planned, 5 summer students enrolled, action on advice of External Advisory Committee (EAD).
Years 2 and 3: By Yr 2 one new faculty member appointed in vaccine research (LSUHSC match commitment); in each of years 2 and 3, four new interdisciplinary Center vaccine projects will begin, at least 2 Center postdocs appointed to Center projects on the advice of the CSC, five conference abstracts and six manuscripts based on Center projects submitted, five new R01/R21 grants or equivalent submitted to NIH and/or other funding bodies, at least 1 new NIH or equivalent award and 1 industry-funded research grant awarded, five manuscripts accepted in high-medium impact journals, 5 new summer students enrolled, 4 graduate students and 4 postdocs appointed from investigator's external grant funds, at least 2 new patents submitted, at least 2 out-of-State meeting/major seminar invitations, meetings in place with Institutions/NOBIC/State/Industry concerning possible local dedicated GMP facility manufacture, formative assessment of Center needs and discussions of performance against goals at least twice a year, including ongoing assessment of Core effectiveness, equipment, and staffing needs; action on advice of EAD – CSC to consider strategies to deal with unexpected developments and institute changes as necessary.
Year 4: as in Yrs 2 and 3, plus at least one P01 program and/or COBRE vaccine-related grant (mentoring/project costs for at least 3 junior faculty on R01-type projects with some smaller 2nd tier

projects) submitted to NIH or equivalent, 2 new junior faculty appointed with start-up commitment from Center and Institutions in new Institutional space, IND in preparation for at least two Center clinical trials, formal (funding) or informal (planning) linkages established with industrial partners. Year 5: as in Yr 4, plus IND in preparation for at least two Center clinical trials; award or resubmission of P01 and/or COBRE vaccine-related grant(s).

(5) SUSTAINABILITY

We realize the importance of planning for sustained growth and development of the Center beyond the life of the P-KSFI award. We would expect to leverage this funding in a ratio of at least 1:1 by the end of the 5-year funding period based on several factors including: (i) the outstanding record of this group of investigators in leveraging Federal and other awards from initial State support, (ii) the level of industrial interest in high quality research in this area, (iii) the traditionally high level of public interest and support for research in vaccines and therapeutics for infectious disease, and (iv) the high degree of potential for development of new intellectual property in this area allied with our collective track record to date. We are confident that we can leverage the initial P-KSFI investment into new funds from federal agencies, private foundations and industry. Multiple avenues for potential funding exist through RFAs issued by NIH, Dept of Defense and Private Foundations (Appendix). These will be vigorously pursued during and beyond the initial 5-year funding period.

We are also confident that research based on our existing funding, on the P-KSFI award, and on further funding opportunities as outlined in the box above, will generate new IP leading to patents and commercial licensing opportunities. The P-KSFI establishment of the *Center of Excellence in Vaccine Development* will, in this way, facilitate eventual stand-alone status, independent of further direct State operating support. We will seek further commitments in terms of space and Faculty start-up packages from participating institutions with our ongoing development. The success of the Center will clearly benefit all three participating institutions in terms of their research profile and funding levels, and the attraction of outstanding new faculty recruits and support staff.

(c) Leveraging of Resources

The State of Louisiana has invested solidly in research and education projects centered on greater understanding of infectious disease. Funds available through the Health Excellence Fund (HEF) have greatly helped to establish projects that address these critical public health issues along with the underlying science. Consortium members have won awards under major State-funded initiatives in this field that have subsequently been leveraged into NIH-funded Programs or Centers in national competition. Prominent examples are the:

1. Gulf South Sexually Transmitted Infections/Topical Microbicide Co-operative Research Center (U19: Martin PI/Quayle/Fidel/Pincus) arose from the State HEF Program: Louisiana Sexually Transmitted Diseases Research Center (Martin PI; 2002-04);
2. Host Defense against HIV-Related Infections Program (P01:Shellito PI, Ramsay, Mason, Reiser) arose from the State HEF Program: Center for Lung Biology & Immunotherapy (Shellito PI;2000-05);
3. NIH COBRE Training Program: Mentoring Oral Health in Louisiana (P20: Fidel PI, Luftig) grew from the HEF Program: Molecular Science of Cranofacial Biology, Infection & Mucosal Immunity (Fidel PI; 2000-05).

In addition, the current HEF-funded, multi-institutional Program: Center for Gene Therapy for Acquired & Genetic Diseases (Ramsay PI, Reiser, Shellito) has, to date, led directly to two new NIH research grants: Vaccination Strategies against Pulmonary Tuberculosis (R01:Ramsay PI, Mason) and Alcohol & Reactivation Tb (R01:Mason PI, Nelson).

This is an impressive record of leveraging State funds into major competitive national research awards and scientific mentoring/research awards, a key factor in the future development of any successful local center of excellence. In the examples listed above, $14M of State funding has led directly to over $32M in new Federal funds with strong probabilities for their renewal.

The interdisciplinary approaches and qualities that our researchers bring to bear within the new focus provided by the *Center of Excellence for Vaccine Technology* will enhance our capacity to win for the State further large-scale funding that is dedicated to progressing R&D in vaccine development (eg. NIH and other Government agencies, Bill & Melinda Gates Foundation, see box above that lists some potential funding sources). Our field of endeavor also positions us well to win funding from industrial sources, including the pharmaceutical industry. Large multi-disciplinary groups such as ours have distinct advantages when competing for funding of this magnitude and our track record is a strong pointer to continued success. The establishment of a Center through the P-KSFI would thus build logically on the outstanding achievements of this group with significant downstream benefits for education, research & development, biotechnology, and public health in Louisiana.

The recent establishment of the New Orleans BioInnovation Center (along the lines of the successful Louisiana Emerging Technology Center in the State capital Baton Rouge), primarily to promote and assist with commercialization efforts coming out of local Universities will aid the progress of the Center towards self-sustainability **[7]**. Their mission is to provide support and guidance by supplying business support services, access to capital and intellectual property professionals, and contacts with industry professionals. Since New Orleans researchers historically attract over two-thirds of the NIH research funding that comes into Louisiana, even post-Katrina ($120M of approx $165M in the first 10 months of 2006; a significant proportion won by investigators in our Center), it follows that the potential for biotech growth is greater here than elsewhere in the State and augurs well for the development of the proposed downtown biotechnology sector around the NOBIC site.

The success of our enterprise will ultimately necessitate access to a Good Manufacturing Practice (GMP) facility that will be required for the preparation of clinical grade material for administration to humans, initially in clinical trials. While the State currently has plans to build a 10,000 sq. ft. GMP facility in New Orleans through the Louisiana Gene Therapy Consortium, this will be dedicated in the first instance for manufacture of adult stem cell and vector preparations that are likely to be incompatible with many of the products envisioned in a vaccine manufacturing facility. It is also unlikely that this facility will be of sufficient scale to meet the needs of both groups. A purpose-built GMP facility that will cater solely for products related to vaccine manufacture in the *Center of Excellence for Vaccine Development* would give us an enormous advantage terms of immediacy of access for manufacture of our products for testing. Currently, those conducting clinical trials in this area often must wait months for a "open slot" at one of a relatively small number of facilities in the US. The exciting possibility that a vaccine-dedicated GMP facility associated with the Center could also function as a contract manufacturing facility that is 'open for business' raises the likelihood of generating a substantial flow of sustaining funds for the Center. It is likely that a stand-alone facility could be built for around $2M with annual operating costs being at least that again. Initial support would be sought from the State and the participating Center institutions. However, a business plan developed by consultant firm BioAbility (Research Triangle Park, NC) for the above mentioned LGTRC GMP facility predicted that it would begin to turn a profit by its 4^{th}-5^{th} years of operation at their anticipated uptake rates. While a similar consultation would be made at the appropriate time in the case of any Vaccines Center GMP, such a facility would have major advantages for our Center and would add considerably to the development and profile of the nascent biotechnology industry in New Orleans.