

Pediatric Tropical Medicine at Baylor College of Medicine

About Us

The Section of Pediatric Tropical Medicine is dedicated largely to the research of neglected tropical diseases and emerging infectious diseases. The section does basic, clinical, and translational research to complement the educational, clinical, and policy-related activities of the National School of Tropical Medicine.

Our vaccine research and development activities are carried out at Texas Children's Hospital Center for Vaccine Development.

The Section of Tropical Medicine Laboratories conduct research in tropical medicine including implementation of new diagnostic tools for NTDs, discovering and mapping the structural design of NTD molecules, and a range of other activities surrounding NTDs and related emerging and infectious diseases.

About the Center

Texas Children's Hospital Center for Vaccine Development is dedicated to the discovery and development of vaccines against neglected tropical diseases and other emerging infectious diseases. The center has developed vaccines against hookworm and schistosomiasis that are currently in clinical trials; in earlier stages of development are vaccines against onchocerciasis, leishmaniasis, Chagas disease, West Nile Virus, Chikungunya, Middle-East Respiratory Syndrome (MERS), and Severe Acute Respiratory Syndrome (SARS). In discovery stages, the center is working on a vaccine that would prevent the three major soil-transmitted helminths - Hookworm, Ascaris and Trichuris.

Center Units

See information about each of the following center's units:

The Antigen Discovery and Molecular Biology Unit

The Antigen Discovery and Molecular Biology Unit expresses and purifies recombinant protein antigens for vaccines against neglected tropical diseases and other emerging infectious diseases. The unit focuses on identification and cloning of the molecules secreted by parasites that play important roles in the establishment of parasitism or survival of the parasites in hosts and evaluating their potential as vaccine candidates.

The unit also develops and optimizes the expression platform for each vaccine candidate protein, mainly using yeast, E. coli, or mammalian cell expression systems; makes research seed stocks based on small scale expression yield and solubility; and expresses and purifies recombinant proteins at the small scale for research purposes. The seed stocks are then transferred to the Process Development Unit for large scale production of vaccine antigens. In addition, the Antigen Discovery unit also maintains the hookworm life cycle in hamsters for pre-clinical vaccine trials and drug screening purposes.

Laboratory Facilities

Dr. Zhan's laboratory has extensive capability of molecular cloning and recombinant protein expression using *Pichia pastoris*, *Saccharomyces cerevisiae*, *Escherichia coli*, Baculovirus and mammalian cell culture expression systems, with fermentation capabilities from the 1-20L scale. The facility is capable of molecular cloning, developing and optimizing protein expression and purification processes and immunological assays.

Procedures may include, but are not limited to, PCR amplification, western blot, ion exchange chromatography (anion or cation), hydrophobic interaction chromatography, hydroxyapatite, reverse phase and size exclusion, IMAC and affinity chromatography.

Process Development Unit

The focus of the Process Development group is to develop upstream and downstream processes to support the Antigen Discovery Unit of the Texas Children's Hospital Center for Vaccine Development. After basic R&D has been performed and potential vaccine antigens are identified by in-vitro and in-vivo testing, the goal of the Process Development group is to optimize the expression of the target antigen to maximize protein yield (Upstream development) and to develop purification processes (Downstream development) to maximize recovery and purity of the target antigen in order to support advanced investigations of the target molecules.

To achieve these goals initial small-scale experiments are performed to 1) identify media, feeds and culture conditions to optimize production of the target molecules, and 2) buffer conditions, resins, and capture/chromatographic steps to obtain target molecule in high purity and stability. When a basic production and purification process is developed for the antigen of interest, the process is scaled up to 10-20L to achieve a true manufacturing process. Once the scaled up process has been established, reproducibility runs are performed to investigate the robustness and repeatability of the manufacturing process in order to establish overall expected protein yields to support third-party cGMP manufacture for clinical trial production.

Laboratory Facilities

The Process Development Unit has approximately 450 square feet of dedicated space, plus shared space in Texas Children's Hospital Center for Vaccine Development including a fermentation room equipped with multiple bioreactors capable of supporting cultures from 5L to 20L.

The laboratory is also equipped with Akta Explorer and Akta Purifier chromatography systems as well as two Acta Avant chromatography systems running Unicorn software for downstream purification.

Vaccine Formulation and Delivery Systems Unit

The unit handles all the formulations of Texas Children's Hospital Center for Vaccine Development, turning recombinant antigens into safe, long-term stable and potent vaccines. The main activities of the unit include:

Buffer Screening

Protein stability is greatly dependent on the buffer in which the proteins are dissolved. Therefore, the Formulation Development Unit screens many different buffers and excipients to select the most suitable conditions to ensure optimal protein stability. The screening strategy involves several advanced analytical techniques, such as Size Exclusion (SEC) HPLC, Differential Scanning Fluorimetry, Dynamic Light Scattering, Turbidity Screening, FTIR and Circular Dichroism to help selecting the most optimal buffer conditions. Only buffers and excipients that are generally accepted as safe and approved by the FDA are considered for final formulation. After the buffers and excipients are selected, the stabilized protein solutions are extensively monitored for possible chemical or physical degradation in accelerated stability experiments and long term stability experiments.

This work is done in close collaboration with the Process Development Unit and the Molecular Discovery Unit within the vaccine center.

Adjuvant Selection

To make potent vaccines from recombinant antigens, the formulation generally requires one or more adjuvants to help generate adequate immune responses. The Formulation Unit collaborates with the Preclinical Testing Unit to study and select the most suitable adjuvants for each specific project. The most common adjuvants currently used are aluminum salts, but the research team also looks into emerging immunostimulatory molecules such as GLA, E6020, CpG and Imiquimod.

Delivery Systems

To improve the delivery of antigens and adjuvants to antigen-presenting cells, we are exploring new delivery systems. Part of our research is based on oil-in water emulsions, another delivery strategy is focused on PLGA microparticles. These delivery systems can be exploited to control the Th1/Th2 polarization of the immune response. The unit evaluates the immunogenicity of the advanced vaccine formulations in mice in collaboration with the Preclinical Testing Unit. The recall response of splenocytes to re-stimulation with protein antigen is measured by CD4 and CD8 T cell proliferation and secretion of cytokines by T cells.

Laboratory Facilities

The Vaccine Formulation and Delivery Systems Unit has a formulation room, plus shared space in Texas Children's Hospital Center for Vaccine Development, including a chemical alcove and a tissue culture room.

The major equipment includes the Alliance and Waters HPLC system, a Wyatt HPLC Light Scattering Detector, a Spectra M3 Plate Reader, a Bio-Rad GS 800 Densitometer, Jasco Circular Dichroism, Bruker FTIR spectroscope, Charles River Endosafe PTS system and a UPLC Waters Acquity, Malvern Zetasizer

ZS90, Labconco Triad freeze dryer, Buchi Rotary Evaporator, PowerGen homogenizer, digital probe sonifier, BioTek plate washer, EVOS digital inverted fluorescence microscope.

Pre-clinical and Immunological Testing

The pre-clinical and immunological testing unit works to test efficacy, immunogenicity, and monitor long-term potency of candidate recombinant vaccines targeted at preventing neglected tropical diseases such as Hookworm, Schistosomiasis, Chagas disease, leishmaniasis, ascariasis, and trichuriasis.

The unit works closely with the Antigen Discovery, Formulation, and Quality Control within the Texas Children's Hospital Center for Vaccine Development as well as in collaboration with other institutions including The George Washington University, the National Institute of Allergy and Infectious Diseases, and the Autonomous University of Yucatán.

Laboratory Facilities

The unit shares laboratory collaborative laboratory space in Texas Children's Hospital Center for Vaccine Development.

The unit also utilizes space at the Center for Comparative Medicine's Feigin Center and Taub Facilities located in the Texas Medical Center.

Common techniques used are multi-parametric flow cytometry, Luminex®, ELISA, and ELISPOT.

The Analytical Development Unit

The Analytical Development Unit focuses on development and improving assays that are supporting the process development and quality control unit. The unit is primarily involved in developing methods that can be used to evaluate identity, purity, and stability of the target products and that can be used as molecule stability indicators. The unit focuses on the characterization of molecules, degradation pathways and defines critical quality attributes in a phase appropriate context. The unit is involved in process comparability studies and coordinates analysis of in- process and product related impurities as needed.

Among the methods used are reverse phase HPLC, size-exclusion HPLC, SDS-PAGE, and Dynamic light scattering. The unit works also with academic collaborators to design and implement novel assays which can aid in determining the best formulation conditions for stability of drug substances and drug products developed by the program.

Laboratory Facilities

The Process Development Unit has a formulation room, plus shared space in Texas Children's Hospital Center for Vaccine Development.

The major equipment includes the Alliance and Waters HPLC system, a Wyatt HPLC Light Scattering Detector, a Spectra M3 Plate Reader, a Bio-Rad GS 800 Densitometer, Charles River Endosafe PTS system and a UPLC Waters Acquity.

Quality Control Unit

The Quality Control Unit is directly involved in the release and stability testing of all vaccine products in development at Texas Children's Hospital Center for Vaccine Development. Quality Control works in direct collaboration with the Analytic Development Unit using qualified assays to evaluate purity, stability and identity of the target products. This involves biophysical characterization of the antigen, evaluation of stabilizers, investigation of antigen interactions with adjuvants, evaluation of product contact materials, and monitoring stability both in real time and under accelerated conditions. Because the vaccine targets often thrive in tropical areas of high poverty, there is a need to ensure that the vaccine formulation can be stable for a relatively long period of time on the shelf once in the clinic.

The Quality Control Unit is currently implementing new assays that can be used to evaluate the stability of the product in tropical conditions. Recent work has focused primarily on hookworm vaccines. The Unit has been responsible for performing all stability testing of the Na-GST-1 protein and Na-APR-1 protein. The unit is also involved in a schistosomiasis vaccine project as well as Chagas disease, MERS, and SARS development projects.

The Quality Control Unit also collaborates with third-party manufacturers during manufacturing prior to tech transfer.

Laboratory Facilities

The Quality Control Unit shares laboratory collaborative laboratory space in Texas Children's Hospital Center for Vaccine Development.

Other Laboratories within the Section of Pediatric Tropical Medicine at Baylor College of Medicine

The Pediatric Section of Tropical Medicine conducts a comprehensive research and development program for producing a new generation of drugs, diagnostics and vaccines for the Neglected Tropical Diseases and Neglected Infections of Poverty, as well as fundamental and applied research against these diseases. To carry out this research, the section's laboratories are organized into these groups:

Zoonotic and Viral Diseases

The Laboratory for Zoonotic and Viral Diseases is primarily focused on clinical research of human vector-borne diseases and emerging pathogens. The unit maintains BSL-2 and BSL-3 laboratories. Current activities include the following clinical/research projects:

West Nile Virus

The Laboratory for Zoonotic and Viral Diseases is conducting a long-term study regarding the long-term effects of chronic West Nile virus infection. Since 2002 the study has followed a cohort of more than 200 West Nile virus survivors with the goal of gaining insight into the long-term renal and neurological effects of West Nile virus. This longitudinal study is currently supported by a National Institutes of Health RO1 grant, a shared U19 grant in collaboration with Yale University, and the Gillson Longenbaugh Foundation. The research study includes annual renal evaluations, and neurological evaluations including neurological exams, MRIs, nerve and muscle studies, and lumbar punctures of persistently infected West Nile patients.

Prevalence of chronic kidney disease and progression of disease over time among patients enrolled in the Houston West Nile virus cohort (open access) (PLOS One)

Chagas Disease

Our laboratory is working to better understand the transmission dynamics and epidemiology of autochthonous Chagas disease in Texas. We have ongoing Chagas studies including analysis of vector distribution, seroprevalence in reservoir animals, screening of high-risk populations, and study of the etiology of cardiomyopathy in Texas. See more information about chagas disease and our studies.

Epidemiology of Neglected Tropical Diseases in Texas

Our laboratory has other projects focused on the epidemiology of neglected tropical diseases in southeast Texas. Working with Dr. Flor Munoz-Rivas, we have developed a predictive severity model for pandemic H1N1 influenza that we are currently in the process of validating with data from patients of the 2013 outbreak. We are performing a toxoplasmosis chart abstraction study to understand the emergence of pediatric cases at Texas Children's and its implication for future disease outbreaks. We are collaborating with Dr. Julie Boom and Leila Sahni at Texas Children's Hospital to perform a surveillance project screening febrile children in the Emergency Department for Chikungunya, Dengue and West Nile virus infection. We currently have enrolled over 900 children into this febrile surveillance cohort. Lastly, we are working Timothy Erickson to meta-analyze murine typhus pediatric cases in the state of Texas and assess its potential as an important locally emerging neglected tropical disease.

Kidney Disease of Unknown Etiology

Since the 1990's, an epidemic of acute kidney failure in Central America has been affecting mostly young, healthy sugarcane workers who lack typical risk factors for kidney disease. Despite a large number of deaths (approximately 20,000 people have already died from this disease) the etiology remains a mystery. Based the patterns of this disease, its clinical characteristics, and what we have observed of the local environment, we hypothesize that an infectious organism could be at the root of the outbreak. Since 2014, Dr. Rebecca Fischer has been leading efforts to collect samples from people, key animal species, and the environment at our research site in Nicaragua in order to look for evidence of infectious pathogens. Understanding the agent-host-environment model of this disease is central to our investigation as we look for interventions and treatment strategies to interrupt this unrelenting epidemic of acute kidney failure.

Structural Biology and Protein Characterization

The structural biology laboratory characterizes the structures and functions of macromolecules that are being investigated as putative vaccine candidates. We develop improved methods for recombinant expression, purification, solubilization and crystallization of diverse proteins. We provide in silico analysis of protein structure/function to guide method development by other units and external collaborators. We also perform biophysical, biochemical, and functional analysis of putative vaccine candidates in collaboration with other units. Our active projects include structural characterization of vaccine candidates, as well as structure-based-drug development for bacterial, parasite and mammalian proteins.

Clinical Parasitology and Diagnostics

The Laboratory of Clinical Parasitology and Diagnostics is dedicated to improving understanding and treatment of parasitic infection through improving systems of their diagnoses. The laboratory's goals are to further define the prevalence and parasitic burden in endemic populations and to help healthcare workers in the field diagnose cases more accurately and more quickly while keeping the cost of diagnosis at a reasonable level, with goals of eradication and improvement in healthcare outcomes.

Laboratory projects include the following:

Molecular Diagnostics of Gastrointestinal Parasites

Because gastrointestinal parasites often thrive in areas of high poverty, there is often a lack of resources for diagnosis, leading to underestimation of the disease burden and in turn to lack of treatment. The Laboratory of Clinical Parasitology and Diagnostics is currently implementing new technology that can be used even in relatively resource-limited areas. In one study, the laboratory is using a testing platform designed by laboratory director Dr. Rojelio Mejia and his colleagues during his time at the National Institutes of Health.

The platform involves quantitative polymerase chain-reaction (qPCR) that evaluates the DNA of a stool sample much more accurately and quickly than traditional microscopy methods. The study involves integrating qPCR testing with microscopy testing for intestinal parasites including *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium parvum* in the relatively resource-limited areas of Quinindé, Esmeraldas, Ecuador. Read more about the study in an article recently appearing in the American Journal for Tropical Medicine, "A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations."

Serological Diagnostics for *Strongyloides Stercoralis*

Since patients may be infected with *Strongyloides* and not have larvae in the stool at the time of testing, for a complete diagnosis of parasitic infection, serological testing is needed in addition to molecular testing at the DNA level. For detection of strongyloidiasis, we will also employ the use of a *Strongyloides stercoralis* ELISA using a recombinant protein to look for IgG *Strongyloides* specific antibody in serum. *Strongyloides* is an auto-infectious parasite, with intermittent shedding of ova/larvae into the stool.

Read more about *Strongyloides* here: "Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by *Strongyloides stercoralis*" by Dr. Rojelio Mejia and Dr. Thomas Nutman.

Vector Biology and Bacterial Pathogens

The Laboratory for Vector Biology and Bacterial Pathogens has three focus areas:

Utilizing Molecular Tools and Animal Models to Decipher Mechanisms of Tick Colonization and Transmission of Spirochetes

This project builds on recent work reporting tick transmission of *Borrelia turicatae*, a species of relapsing fever spirochete. The study suggested that once the bacteria colonize tick salivary glands they are preadapted to enter the mammal. Our goal is to understand how relapsing fever spirochetes colonize the tick and are transmitted to the mammal, in order to identify targets for a vaccine against the pathogens.

Surveillance of Pathogens Transmitted By Soft Ticks

Soft ticks not only transmit relapsing fever spirochetes but also African swine fever virus, an emerging and highly contagious pathogen with high mortality rates in domestic pigs. Through collaborations with Texas A&M, the USDA, and the Universidad del Valle de Guatemala, we utilize diagnostic assays to evaluate mammalian exposure to soft ticks and the pathogens they transmit. These projects will define the disease burden in regions of the globe where the pathogens are ignored.

Investigating the Ecology of Soft Ticks

This study is to investigate soft tick distribution and maintenance in nature, with the long-term goal of understanding vector dispersal.