important biological role and a potential impact on biomedical research. This is a service center and investigators can submit potential diagnostic protein target for analysis.

In 2008, NIAID proposed a comprehensive Research and Development program that spans from identifying specific targets to developing new and improved technologies for sample preparation and processing, platform development, enhanced detection methods, clinical validation and data acquisition. This was achieved through a variety of NIAID award mechanisms. The results are a comprehensive diagnostic program that spans a broad spectrum of projects from emerging technologies, such as nanotechnology, to further advanced product development of established technologies and platforms for US government diagnostic preparedness.

In terms of clinical specimens for TB research, NIAID can provide access to samples in addition to those available through WHO and FIND. NIAID also can provide leprosy clinical specimens.

Dr. Beanan also provided a summary of NIAID funding opportunities in diagnostic development. These are listed at the end of this document. She briefly described the programs that are available to academia and industry. She noted that the Cooperative Research Partnerships for Biodefense is a large award involving multidisciplinary teams and encouraging partnership with industry.

She concluded by indicating that NIAID DMID has a specific web-page relating to diagnostics.

NIBIB/NIH Resources for Diagnostic Developers:
John Haller, Program Director, Liaison for International Activities

Dr. Haller provided a brief overview of the organization of NIH and its placement within the Department of Health and Human Services in the Executive Branch of the federal government. He noted that NIH is composed of 27 institutes and centers, each of which has different missions, budgets and ways of deciding funding priorities. The focus of his institute, the National Institute of Biomedical Imaging and Engineering, is applying engineering and physical sciences to the life sciences.

The NIH supports biomedical research using a number of different approaches. These include multi-disciplinary approaches, which involve applying principles and methods from the physical and quantitative sciences, and engineering to address problems in biology and medicine and partnerships and collaboration, which involve multi-disciplinary and multi-organizational teams. More recently, NIH has placed more emphasis on design and technology-driven research in addition to the traditional clinical hypothesis-driven research. There has also been more emphasis on technology transfer, that is, moving discovery to patients and to products (bench-to-bedside-to-practice). The NIH Award mechanisms include research projects, institutional training grants, individual training awards (fellowships) and career development awards. The research project awards include the traditional investigator-initiated R01 awards (about $300K/year for 4-5 years) and the exploratory and small grant awards, the R21 award ($275K total over two years) and R03 award ($50K per year over 1-2 years), respectively. He noted that most NIH-research is unsolicited. Thus, investigators do not have to wait for solicited research such as program announcements (PAs) and request for applications (RFAs). Each NIH institute has its own web-site (which can be accessed through the NIH-home page, www.nih.gov, or directly through its specific URL. Information on NIH solicited grants can be found at http://grants1.nih.gov/grants/guide/. This site also allows one to sign-up for e-mail notification about funding opportunities.

Among the NIBIB program areas that are most relevant to diagnostic development are those focusing on sensors and on micro-and nano-system; platform technology. The focus of the Sensors Program is on the development of sensor technologies for the detection and quantitation of clinically relevant analytes. The focus of the Platform Technologies Program is the development of BioMEMS, microfluidics and nanoscale technologies, including micro-total analysis systems, arrays, and biochips, for detection and quantitation of clinically relevant analytes in complex matrices. The NIBIB program officer for these programs is Brenda Korte, Ph.D. (kortebr@mail.nih.gov).

involved the purchase of the IP for a phage replication assay for detection from a small company because of concerns about the company's viability. The third example is the effort with Dr. Alland and Cepheid on a TB POC diagnostic, which is the focus of another presentation of the meeting.

Dr. Perkins illustrated the criteria by which FIND classifies projects as feasibility, evaluation and demonstration. He noted that the factors influencing the cost of trials include: whether they are cross-sectional versus longitudinal; the type of clinical information needed, the nature of the required gold standard (e.g. blood culture, drug susceptibility testing), the specimen needs (e.g. serum) and volume; the nature of the patient groups (pediatric TB, extrapulmonary or disseminated TB, contact persons) and whether other donors/partners are involved (e.g. CREATE, AERAS, Universities, MOH).

NIAID/NIH Resources for Diagnostic Developers:
***Maureen Beanan*, Program Officer, Genomics Program, National Institute of Allergy and Infectious Diseases, NIH, DHHS**

Dr. Beanan indicated that the NIAID mission is to support "basic and applied research to prevent, diagnose and treat infectious and immune-mediated illness, including HIV/AIDS, and other sexually transmitted diseases, illness from potential agents of bioterrorism, tuberculosis, malaria, autoimmune disorders, asthma and allergies." NIAID provides a full range of resources and support to investigators across the spectrum from basic research to the development of therapies, vaccines, and diagnostics.

Dr. Beanan provided information about the NIAID resources and support related to the development of medical diagnostics. The NIAID "omics" resources are especially relevant to target identification. NIAID supports two Microbial Genome Sequencing Centers that have the goal of rapid and cost-efficient production of high quality sequences of human pathogens and invertebrate vectors of disease. The two centers accept requests from the scientific community, but not from individual investigators. Once the sequences are available, NIAID provides support for their annotation and tools for analysis through eight Bioinformatics Resource Centers. These Centers have the goal of providing the scientific community with a robust point of entry for access to genomic and related data in a user-friendly format. Of these Centers, there is one that supports analysis of TB data.

The NIAID Pathogen Functional Genomics Resource Center has the goal of developing and distributing genomic reagents, resources and technologies for the functional analysis of pathogens and invertebrate vectors of disease. This Center can provide organism-specific microarrays and protocols, protein expression clones, genotyping and genome analysis and the development of computational tools for array and comparative genomic data analysis.

The goal of the NIAID Population Genetics Analysis Program is the discovery and analysis of human genetic variations (polymorphisms) associated with susceptibility or resistance to infection and/or response to vaccination. The diseases under study include: West Nile Virus, tuberculosis, influenza, and encapsulated bacterial infections. The vaccination responses being investigated include those to: *S. typhi*, *V. cholerae*, *B. anthracis*, and smallpox.

The focus of the seven NIAID Proteomics Research Centers is to characterize the pathogen and/or host cell proteome, identifying proteins associated with the biology of microbes, the mechanisms of microbial pathogenesis, and the host response to infection. Each of the Centers specializes in specific pathogens and uses technologies such as NMR and x-ray crystallography, yeast-2-hybrid analysis, mass spec analysis and nucleic acid programmable microarrays. All of the data and technologies from the Centers are made available on a centralized resource web-site. However, they are not open for collaborations.

The two NIAID Structural Genomics Centers for Infectious Diseases apply state-of-the-art high-throughput structural biology technologies to experimentally characterize the three dimensional atomic structure of targeted proteins from pathogens in the NIAID Category A-C priority lists and organisms causing emerging and re-emerging infectious diseases. The primary focus is on pathogen targets that are expected to have an

and includes: (1) name (nonproprietary name and brand name); (2) purpose of use; (3) configuration, structure and principle; (4) ingredients involved in reaction system; (5) specifications; (6) operation procedures or instructions for use; (7) manufacturing methods; (8) instructions for storage and shelf life; (9) Information on manufacturer; and (10) information on manufacturer of drug substances. The data that needs to be included is similar to that which one would submit for a 510(k) or PMA and include: (1) data concerning the origin and background of the discovery, use in foreign countries, etc.; (2) data concerning specifications; (3) data concerning stability; (4) data concerning performance; (5) data concerning risk analysis; (6) data concerning the manufacturing method; and (7) data concerning clinical performance. Basically, data are needed to show the conformity to standards and essential principles.

Overall, the key points in review of an IVD are: Is the function appropriate? Is the IVD pertinent to the clinical significance of the item to be detected? This is especially important when a new clinical significance needs to be proven for new or existing items.

There is also a process for a Raw Data Check, which is equivalent to a data audit in the U.S. The targets of the Raw Data Check are new items and existing items that have new clinical significance. The purpose is of the Raw Data Check is the confirmation of the scientific reliability of the data and the confirmation of the contract with the study sites, including the confirmation of informed consent from patients.

There are also pre-approval inspections. These are tests that are considered to be necessary by the MHLW before giving marketing approval. They are conducted by the designated research organization (National Institute of Infectious Diseases [NIID]) for the IVDs that are considered to be important in light of public health. A sponsor needs to contact the agency to get a sense of current policy relating to specific infectious agents.

SESSION 3: From Bench to Bedside

Overview of the Issues:
***Steven Reed*, Head of Research and Development,**
Infectious Diseases Research Institute (IDRI)

Dr. Reed stated that he would summarize some of the issues he has dealt in the development of diagnostics, particularly for TB, Leprosy and parasites (Leishmania, Chagas', etc.). He noted that the early and accurate detection of infection can lead to early therapy, novel therapy, reduced time-to-cure, and reduced transmission. Thus, there are both clinical and public health impact to having good diagnostics. Moreover, diagnostics can be used as tools for follow-up of patients and to test for cure of the infection by measurement of biomarkers. For TB and Leprosy, the treatment protocols are long and there is often poor compliance with therapy. Having good diagnostics could lead to better compliance.

There are a variety of types of diagnostics that can be used to detect infection, including: those for antibody detection in blood, plasma, serum, which are indirect measures; those for antigen detection of protein, of DNA, or of RNA, which are direct measures; and those for detecting T cell responses, which are also indirect measures. Among the issues that need to be resolved for developing an effective diagnostic is the need to find the ideal antigens for indirect tests and the ideal antibodies for direct tests. Another key issue is having a good format by which to implement the assay. Among the complexities of disease that need to be considered in developing diagnostics are the low antibody levels early in infection and the heterogeneity of the infectious organism. For example, in TB, any given antigen can only detect 30-40% of infected individuals; thus, there is a need to combine antigens for a TB diagnostic.

Factors that need to be considered in the product profile of a diagnostic include: accuracy (sensitivity, specificity); a design that is low tech, and easy to use in the setting of a simple-laboratory; an assay that is rapid so as to enable real-time therapies, that is while the patient is still in the clinic or office; an assay that is semi-quantitative, e.g. a visual assay, or if quantitative can use inexpensive, high performing readers. The level of performance may also be dictated by whether the assay will be used for diagnosis versus for blood bank screening; the latter usage requires a high performance assay.

Factors that should be considered in implementing an assay are: accessibility, distribution; copies of the assay/infringement, engaging manufacturers (patent costs, development costs, etc.), the licensing strategy and the paths forward (SBIR funding, regulatory approval, etc). Dr. Reed indicated that his organization uses only non-exclusive licensing. He noted that most diagnostic companies have their own format for an assay that works to the manufacturer's advantage. He indicated that in the developing world there are situations in which there is a "copy-cat" assay that does not perform well but which uses the same name as a bona fide product. In terms of covering developmental costs for diagnostics for developing world use, there are a few companies that have provided assistance to IDRI as well as non-profits, such as, the Bill and Melinda Gates Foundation (BMGF). NIH SBIR support has also helped IDRI in the development of tests for Leishmaniasis, TB and Chagas'. In terms of regulatory approval, the neglected disease status has been emphasized when working with the FDA.

Dr. Reed described the product profile of a test for African Leishmaniasis that IDRI developed with assistance first from an SBIR and then from the BMGF. This is a small volume ambient temperature test. It is intended for the semi-quantitative measurement of antibody response following *Leishmania* infection and for the laboratory diagnosis of visceral leishmaniasis when used in conjunction with clinical information.

Role of Animal Model Data in Developing Diagnostics:
***Ray Waters*, National Animal Disease Center,**
US Department of Agriculture

Dr. Waters discussed the opportunities for using veterinary models for the development of human diagnostics. He noted that from the animal husbandry point of view, it is important to eliminate bovine tuberculosis in the U.S. *M. bovis* causes bovine TB and has a wide host range. In the U.S., white-tailed deer (WTD) are among the primary hosts. Bovine TB was almost eradicated in the U.S. with only pockets of infection remaining (e.g., Michigan, Minnesota, New Mexico, California). Current cases are associated with imports from Mexico and transmission from free-ranging WTD in Michigan.

A number of features make bovine TB a good model system for the development of human diagnostics. There are aerosol challenge models of *M. bovis* in the U.S., U.K and New Zealand that use the natural outbred host and offer the potential to study disease in the neonate, the role of nutrition in disease, and the role of non-TB mycobacterial infections. In addition, there are opportunities for field trials in high prevalence areas such as Mexico as well as the potential to study interactions with other infectious diseases in these areas. There is also the possibility to study TB infections in zoo animals, such as elephants. Bovigam, an interferon-γ-release assay has been developed in collaboration with Prionics AG in Zurich. Dr. Waters presented examples of data using this assay.

In terms of control of *M. bovis*, it has been found in field studies in Michigan that supplemental feeding results in high deer numbers, due to decreased winter death loss, crowding around feed sites, contamination of feed by *M. bovis*-infected animals, stress due to crowding, and increased

risk of disease transmission. An initial strategy in Michigan was to have less baiting of deer, and therefore less crowding, and to allow for more hunting. While this helped, it did not eliminate disease. The new strategy for control is to test deer in the field, destroy infected animals and vaccinate uninfected animals. The work is being done in collaboration with the Michigan Department of Natural Resources and Chembio. Since hunters have to send in the heads of deer, it is possible to monitor disease in this way. More than 153,000 heads have been tested by MSU and the Michigan Department of Health; 568 of them were *M. bovis* positive. The specificity of rapid diagnostic tests using whole blood under field conditions is quite good (>99%); the sensitivity is comparable to other tests (in the 55% range) and may reflect the extreme field conditions under which it is performed.

Dr. Waters concluded by suggesting that the next great discovery for the control of human TB may be tested first with cattle.

CLIA Regulations: The ABCs of Validating a Diagnostic for Infectious Agents:
***Christina Egan*, Director, Biodefense Laboratory, Wadsworth Center**

Dr. Egan stated that she would address the CLIA regulations and their application to the validation of an infectious disease diagnostic from her perspective at the Wadsworth Center. The Wadsworth Center is the research-intensive laboratory of the New York State Department of Health. It is a unique blend of basic and applied research and also includes reference-level clinical services. Her laboratory is involved in biodefense efforts as well as in the clinical laboratory-public health issues.

The Clinical Laboratory Reference System (CLRS) was established in 1964. The CLRS system includes: inspection of laboratory facilities and distribution of proficiency test specimens for laboratory examination, or other measures of laboratory performance; validation and approval of standard laboratory methods and materials; and cooperative research relevant to advancement, development and assessment of laboratory methods and materials. Any laboratory that wishes to perform a molecular assay must submit an application for evaluation and approval. There are submission guidelines and sample submission documents available. NYS Reference System scientists have reviewed more than 3000 SOPM and validation submissions for laboratory developed and modified FDA-cleared assays for their intended use. For test approval, submitting laboratories must establish analytical and clinical validity.

All clinical laboratories in New York State (NYS) are required to be permitted through the Clinical Laboratory Evaluation Program (CLEP). Any laboratory (including the Wadsworth Center or out-of-state facilities) wishing to perform a molecular assay must submit an application for approval to use it on patient specimens from New York State.

For nucleic acid amplifications tests for infectious diseases, the submission requires sections on: methods; requisition and reporting; references; validation protocol and data; and quality assurance. Dr. Egan provided a summary of the validation process for molecular assays. This involves the establishment of performance criteria for an accurate measure of assay quality and includes such aspects as: *in-silico* development (target gene, database search, primer optimization, probe optimization, Mg+ optimization, sensitivity (Determination of LOD with and without matrix), specificity, accuracy, precision, range and robustness. Assay development is an iterative process of assessment and improvement. There should be a clearly identified goal for the assay, e.g. for basic research versus as a clinical diagnostic or as a qualitative versus a quantitative assay.

With respect to the validation process, the NYS CLEP Guidelines for Test Approval for Molecular Assays require a developed validation plan, and an assessment of sensitivity (Determination of LOD with and without matrix), specificity, accuracy and precision. Dr. Egan

presented examples of the types of testing and assessments that her laboratory had done in the development of assays for *Francisella tularensis* as part of her involvement in biodefense research.

Dr. Egan presented several examples involving the validation of Home Brew Assays. To assess accuracy of such assays, one must conduct a blinded study in one of the three following ways: (1) comparison of the results obtained to a gold-standard or FDA-approved assay; comparison of the results obtained to the results from another CLEP approved assay; or use of spiked clinical samples and comparison of the experimental results to the expected results. There must be at least 30 positives samples and at least 10 of the samples must be close to or no more than ten-fold over the limit of detection (LOD). The test must be performed in the appropriate matrix. If no clinical samples are available, seeded specimens can be utilized

Dr. Egan noted that another important CLIA consideration is the use of appropriate controls. These include extraction controls (Bacteria or virus is preferred; if these are unavailable one can use DNA. The agents should be seeded at a low level.); amplification controls; and inhibition testing controls. She noted that with respect to inhibition controls, many laboratories do not test for them or test for them improperly. Ideally, inhibition controls are used as an internal control in every sample. For a well-established method, NYS allows for the submission of data from 100 samples per specimen type.

Dr. Egan noted that platforms may change during the development of an assay and that there is a need to revalidate the assay with respect to the new platform. CLIA also requires the validation of assay modifications. Such modifications include: change of intended use, use of sequence databases for identification of agents, and use of multiples analytes.

SESSION 4: Role of Partners

WHO's Global Contribution to Improved TB Diagnostics:
Rosanna W. Peeling, Manager, Diagnostics R&D, UNICEF/UNDP/WorldBank/WHO Special Programme

Dr. Peeling stated that she would address the diagnostics landscape in the developing world from the perspective of the STOP TB Department and the Accessible Quality-assured Diagnostics, programs at WHO/TDR which are partnerships designed to move TB diagnostics forward.

She noted that although high-quality diagnostics are available for many infectious diseases, they are neither affordable nor accessible to patients in the developing world. There is little industry interest in developing quality diagnostics for diseases prevalent in the developing world due to a perceived lack of return for investment. The lack of regulatory control on diagnostics has resulted in tests being sold and used in the developing world without evidence of their effectiveness; this factor also discourages companies with good products from competing in the developing world market. An additional impediment is the lack of norms and standards on how to assess the quality of diagnostics in the developing world.

As an example of some of these issues, Dr. Peeling presented data on the performance of Dengue rapid tests, comparing manufacturers claimed accuracy with the much lower performance found when the WPRO tested the assays.

WHO efforts in TB include: The New Diagnostics Working Group (NDWG); The Retooling Task Force; the Global Laboratory Initiative; The Intergovernmental Working Group on Innovation, Intellectual Property Rights and Public Health; UNICEF/UNDP/World Bank/WHO Special Programme on Research and Training in Tropical Diseases (WHO/TDR); the Stop TB

Partnership and the World Health Organization Global Plan to Stop TB 2006-2015.

The WHO/TDR mission is "to foster an effective global research effort on infectious diseases of poverty in which disease endemic countries play a pivotal role." This is reflected in the framework of collaboration between the TDR and Stop TB efforts which is built around developing new and improved control tools so as to obtain more effective approaches for TB control, generating evidence to assist policy-decisions, reducing TB disease burden by increasing diagnostic laboratory capacity in countries, gaining increased support and expanded engagement of partners, and promoting a research culture within national control programs.

Dr. Peeling stated the overall objective of the Accessible Quality-assured Diagnostics program which is to promote and facilitate the development, evaluation and application of diagnostic tests appropriate for use in developing country settings. The specific program objectives are: (1) to define diagnostic needs for diseases of poverty and to set standards for diagnostics quality; (2) to facilitate test development; (3) to assess and assure diagnostic performance and quality; and (4) to increase access to diagnostics in the developing world, taking into account socioeconomic factors and issues of gender equity.

Dr. Peeling illustrated the steps along the pathway from test development to sustainable adoption of the test that the TDR/WHO program can assist the test developer in achieving their goals. This assistance includes definition of the needs and product specification, access to laboratory and field site networks, providing models of impact and cost effectiveness, consultation with national program managers and bulk procurement and monitoring. She provided an illustration of the range of products and their developmental stages for which WHO/TDR is supporting diagnostic R&D sites in 35 countries.

Dr. Peeling provided an overview of the status TB-related activities that are being undertaken as part of the four objectives of the Accessible Quality-assured Diagnostics. With respect to Objective 1, the goals are to: synthesize existing knowledge by conducting systematic reviews and convening meetings to identify gaps in knowledge and define needs; model the impact of diagnostic delay and drop-out; develop guidelines on the design and conduct of diagnostic evaluations; and estimate the human and economic cost of bad diagnostics. To this end, they have commissioned a review that will be published by FIND and will be similar to the reviews commissioned and published in Nature on malaria and sexually transmitted infections. At this point they have not examined the human costs of bad diagnostics, but they have recognized this as a problem and are working with the World Bank to estimate the cost of this problem.

With respect to TB test development activities (Objective 2), they have published information on global demand and product specifications to promote public/private sector engagement, they have funded fund "bright ideas" projects for novel/improved test formats; and though their specimen bank are able to provide well-characterized specimens and strains. The NDWG is working on a Scientific Blueprint for TB Diagnostics Development.

With respect to Objective 3, assessing and assure diagnostic quality, they have evaluated 19 rapid tuberculosis tests using 355 sera from 8 geographically diverse sites and found that sensitivity ranged from 0.97% - 59.7% and that specificity ranged from 53.0% - 98.7%. The test with the highest sensitivity had a specificity of 57.7%. Tests with the highest specificity had sensitivities of 2.4% and 0.97%. In addition, STOP TB and TDR co-convened an Expert Committee Meeting on the use of molecular diagnostics for the rapid identification of MDR-TB, 31st March 2008. Multi-country studies on improving microscopy have been organized. Strains from the TB Strain Bank were used in 13th Round of EQA SNRL.

Work has not yet been started on Objective 4, but they are in the process of identifying models

of other diseases that may be appropriate for use with TB.

WHO's ultimate goal with respect to TB diagnostics is to have a major impact in reducing TB disease burden through disease endemic countries having increased capacity to provide quality-assured diagnostic services at all levels and to conduct their own diagnostics research for policy

FIND's experience with Late Stage Development of Rapid Diagnostics:
***Mark Perkins*, Chief Scientific Officer, Foundation for Innovative New Diagnostics (FIND)**

Dr. Perkins noted that much of the endemic-world diagnosis of TB uses the same technology applied to TB in 1892, light microscopy. Although the costs for developing diagnostics are about 10-fold less than for drug development, the timeline is long and developing countries cannot afford these costs. Although public-private partnerships have been established for reducing the impact of AIDS, malaria and TB by supporting development of vaccines, therapeutics, and diagnostics, diagnostic development has received much less emphasis than the other efforts. He also noted that at either end of the development process, basic research and production-distribution, there are often clear sources for support of the work; the middle part of the developmental stream is an area, which while highly important, does not have sufficient support. Thus, downstream in the diagnostics development process, proof-of-principle studies may be funded by research agencies; and upstream, agencies, donors and ministries of health provide support for public health programs.

FIND was formed to fill this gap in the development process, and to form partnerships with industry to drive development of products meeting public needs. FIND is built on a business model of mutually beneficial partnerships. FIND-partner contracts are a function of the stage of product development and clearly define the roles and responsibilities of each group. Dr. Perkins noted that different products and industry partners may need assistance and advice along the various stages of product development. Thus, companies may need assistance in defining the medical need, obtaining key reagents or reference materials or getting access to clinical sites. FIND also collaborates with WHO in filling gaps in company expertise or needs. In an early phase product concept collaboration, FIND might provide assistance with project management, customer/disease know-how, or trials, while the partner would provide product development and technology know-how. In a mid-phase effort involving product development, FIND might provide assistance in project management, trials or WHO policy, while the partner would be involved in product development, regulatory submission, making the product, and providing customer support. In a late phase effort involving demonstration and supply of a product already "in-the-box," FIND might be involved in project management, trials, WHO policy, acceleration of uptake and measurement of impact, while the partner was involved in making the product, registration, distribution and customer support.

FIND has been involved in managing intellectual property all through the value chain. Dr. Perkins provided examples of how FIND has achieved royalty-free licenses for all sectors in developing countries for products in the feasibility stage and royalty-free licenses for the public and civil society sector in developing countries for products in the development stage. For products in the demonstration-evaluation stage, FIND has gotten pre-agreed affordable prices for public sector and civil society in developing countries.

Dr. Perkins enumerated the multiple factors that go into determining the cost of a diagnostic, which include not only the cost of the goods, but also the cost to provide service, such as training, quality assurance and maintenance of equipment (hardware and software). The cost of providing service can be an additional 20 percent of the cost of the goods. FIND is working to bring the cost of deploying diagnostics down by expanding the market early.

Thus far, FIND has focused on three areas, malaria, sleeping sickness and TB. Dr. Perkins noted that there are 45 sites around the world where FIND is involved in development, evaluation and demonstration projects. FIND has invested in technologies rather than in making grants. With respect to TB, the goal is to develop a platform. He provided several examples of FIND's TB efforts. One involves the Becton Dickinson MGIT test, which is an example of making a US standard available in a developing country. Another example

The NIBIB also supports four Point-Of-Care Technology Centers: Center to Advance POC Diagnostics for Global Health (GHDx), Bernhard Weigl, Ph.D. (Program for Appropriate Technology in Health (PATH)); Center for Point-of-Care Technologies Research for Sexually Transmitted Diseases, Charlotte Gaydos, M.P.H., Ph.D. (Johns Hopkins University); Center for Point-of-Care Technologies for Disaster Readiness, Gerald Kost, M.D., Ph.D. (University of California, Davis); and Point-of-Care Center for Emerging Neurotechnologies (POC-CENT) Fred Beyette, Ph.D. (University of Cincinnati).

Especially relevant to the workshop participants is the GHDx Center which works to improve the availability, accessibility, and affordability of essential point-of-care diagnostic tests for use in low-resource settings around the world. The GHDx Center is a collaboration of PATH, an international nonprofit organization that improves the health of people around the world, and the University of Washington. The Center has four core activities: clinical needs assessments; supporting exploratory technology projects; clinical testing of prototype point-of-care diagnostics; and training on the clinical realities of designing point-of-care products for low-resource settings. The PATH group is seeking collaborations for clinical testing and is also involved in training scientists about clinical realities.

Dr. Haller spoke briefly about policies and mechanisms for NIH awards to foreign institutions. He noted that NIH has specific criteria that must be met for grants to foreign institutions. These relate to a demonstration of "special opportunities for furthering research programs through the use of unusual talent, resources, populations, or environmental conditions in other countries that are not readily available in the United States or that augment existing U.S. resources." A more common mechanism is to have within an award to a domestic institution a sub-contract for a collaboration with a foreign institution. Over the past several years, there has been an increase in research awards and funding to foreign institutions. The NIH is also developing a web-site specifically for foreign investigators. The web-site will provide a range of information including tutorials, information on mechanisms, frequently asked questions and NIH contacts and resources. It is anticipated that the web-site will be operational in December 2008). Dr. Haller also noted that each NIH organization has program officers like him who specialize in international activities.

SESSION 5: Lessons Learned: Case studies of diagnostic development experiences

Tb/Leprosy/Other Mycobacteria

***David Alland*, Chief, Division of Infectious Disease, Professor of Medicine, New Jersey Medical School**

Dr. Alland described his efforts toward developing a commercial assay to detect *Mycobacterium tuberculosis* (TB) and described the lessons he has learned along the way. He indicated that this work began conceptually as part of a fellowship project in 1989. Thus, the first lesson is that the academic timeline is longer than the commercial one and that it is important for researchers to take the long-term view and to be persistent. The need for an assay for multi-drug-resistant (MDR) TB has been emphasized by the large rise in drug-resistant disease. The assay is based on the fact that by definition, all MDR TB is rifampin resistant, and since rifampin-mono resistance is rare, detection of a marker of rifampin-resistance has been proposed as a surrogate for MDR. Rifampin-resistance resides in the *rpoB* gene, and using this information, Dr. Alland developed a molecular beacon assay to detect mutations in the *rpoB* core region. He constructed five molecular probes that cover this genome region. By labeling each molecular (probe) beacon with a different fluorophore, it is possible to perform the entire assay in a single well. The absence of one of the beacons suggests a mutation in a region associated with drug susceptibility, and thus indicates drug resistance. The California Department of Health adopted this assay for their MDR testing.

Dr. Alland was seeking a corporate partner to develop this assay for POC use and discovered Cepheid, a small California company that had a vision of a cartridge-based assay system called SmartCycler. Dr. Alland's second lesson was learning the need to pay his own way to visit the company, educate them and eventually develop a collaboration.

A key impediment to developing a PCR assay for POC testing for TB is the need for a method to process sputum. To develop and test this method, Dr. Alland and Cepheid applied for an SSTR grant. He recognized that the corporate skill set, while high in engineering expertise, was not sufficient for grant writing. His third lesson was that he needed to undertake this task, not only for his portion of the work, but also for the engineering portion. The SSTR funds were used to develop the methodology to process the sputum. During this interval, the company also made progress on the cartridge and machine.

At this point, his fourth lesson was the realization that although it appeared that they were "done," in fact, they were just beginning. In a Phase II SSTR application, Dr. Alland and Cepheid joined with FIND for development of the components into a useful diagnostic.

The fifth lesson that Dr. Alland cited is that the (Design Input Requirements (DIR) are important. FIND and Dr. Alland spent about a year and a half developing these as the Phase-gate Review process will stop the entire project if it does not meet the DIR. Dr. Alland's sixth and seven lessons relate to communication which is key and is hard work. Among the three participating parties, Dr. Alland, Cepheid and FIND, a formal process is in place for weekly conference calls that are accompanied by written reports. Four times a year, the three participating groups meeting face-to-face for 1 to 2 days.

When nested PCR did not improve the limits of detection, it was necessary to go back-to-the-drawing- board and develop: a new PCR enzyme formulation; a new "better performing" PCR assay and assay conditions; and a new formulation of sputum digestant. Working together, the group was able to accomplish this in 2 ½ months.

Dr. Alland's eighth lesson was that in the academic arm of the collaboration, it is possible to make changes and explore tangents, such as investigating the question of whether it was possible to use the sample treatment buffer to reduce biohazards. This was tested and appeared to accomplish this goal.

The ninth lesson is that there are many other activities that you might not have considered. For example, every time a new component is brought in (such as new cartridges), all of the other components need to be retested with the new cartridges. Stability studies must be done and reagents need to be set aside for troubleshooting problems. Error analysis must be in place, that is, if a mistake is made, can it be detected. Reproducibility must be tested and demonstrated. Methods must be in place for verifying all reagents.

The tenth lesson is that every claim needs to be validated and documented. Thus, if one were to decide late in the assay development process that the assay should be semi-quantitative rather than qualitative, an enormous amount of work would be needed. The final lesson is the importance of documentation to ensure that every aspect of the work can be replicated exactly.

Parasites: when an academic derived idea meets development reality.
***Alan Magill,* Director, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR)**

Dr. Magill described the efforts at WRAIR to develop a rapid malaria diagnostic test. This work started in 1994 as a result of the problems with malaria illness at the Army's 86th Evacuation Hospital in Somalia. While a diagnosis of malaria can be made though high quality microscopy, this is not feasible under field conditions in endemic areas. As a consequence, there can be both over diagnosis and over use of drugs as wells as missed disease. The process has been a long one with FDA clearing the Binax NOW® Malaria Test in 2007. The Binax test is a 10 minute test that is non-microscopic, requires minimally trained personnel,

and is environmentally robust.

Dr. Magill traced the process of developing the test through 10 years of prototype testing and trials in Thailand and Peru. These countries were chosen because fixed sites were in place and the population had a low burden of disease, and thus, was similar to the U.S. population. Through this process, Binax, the manufacturer of the approved product, was down selected from other potential sources. Dr. Magill noted that the approval process was a massive exercise in document control and management.

He cited a number of lessons-learned in the development of diagnostic products. He noted that it is important to clearly define the intended use and to consult with people in the intended environment about how a future test might be used. It is important to conduct time and motion studies to demonstrate to the end-users that time and energy will be saved by the test. The clinical benefit and actions that will be taken based on the test result should be clear. The need for a quantitative versus qualitative read-out should be carefully assessed as the former makes product development more difficult.

Other issues that need to be considered are the limitations of the existing health systems, whether clinicians already use a test or would use one if it were available. If there is not a tradition of QA/QC, this has to be built into the diagnostic device. A determination should be made of who would buy the test, whether it is wanted or will be used and who will benefit from its use. One needs to consider who will pay for the development, expansion and manufacturing of the test. It was noted that while a lot of international donors are helpful at accelerating the development of the diagnostics, they are reluctant to become the sustainers of its use.

Defining the critical path, target product profile, and target package insert are key issues to which considerable time should be devoted up-front. It is important to define the key performance parameters, to develop a draft package insert and to follow FDA guidance. Examining the product inserts for recent similar products approved by the FDA should provide insight in this area. The clinical development plan should also be defined up-front. Choice of the comparator method is very important. A decision should be made as to whether regulatory approval will be sought, and if so, from whom. Some products may benefit from being designated as orphan products in terms of tax benefits and FDA funds for clinical trials. Another key decision point is the choice of the co-development partner, a company that is neither too large nor too small, that has an external revenue source and an FDA-approved product. Written agreements should be obtained for all steps. The manufacturing partner can be the same or different entity as the co-development partner. It is important that all the agreements with the commercial partners extend through buy-outs, mergers and consolidations. It is also important to have kick-out clauses for partners that do not meet benchmarks. It is also important to recognize that the skill sets needed for a bright idea and for commercialization are not the same. Projects should be appropriately resourced with a dedicated multidisciplinary team that is exclusively focused on the project and for which milestones decision points and timelines are clearly defined.

Acute Respiratory Infections: challenges with multiplex platforms for diagnostics.
***Ian Lipkin*, Jerome L. and Dawn Greene Infectious Disease Laboratory, Columbia University and Wadsworth Center**

Dr. Lipkin described his work focusing on the identification of new pathogens and determining which of them should be moved forward for development of diagnostics. He noted that they are many factors in emerging and re-emerging diseases including microbial adaptation and change, and globalization. He suggested that new threats will emerge in geographic hot spots and in

hot hosts, e.g. persons involved in processing bush meats or working in abattoirs.

He described a staged molecular strategy that he uses for pathogen discovery and identification. In this strategy, he starts with the least complex assay and then proceeds to more complex strategies in order to generate candidate pathogens. These strategies are: mass tag PCR panels, Greene Chips, and shot-gun sequencing. The final step in the process is showing that the candidate pathogen is related to the disease. Dr. Lipkin notes that the process often begins in the clinic where physicians observe an unusual clinical presentation.

Dr. Lipkin provided several examples in which this approach has been used to identify new pathogens. Working with the New York Department of Health at flu-like illnesses and using the mass tag PCR approach, he identified a clade of rhinoviruses that was as different from other rhinoviruses as rhinoviruses are from other enteroviruses.

Using Greene Chip protocols and a pan-microbial chip, he studied specimens from an NGO worker who had died in Angola during an outbreak of viral hemorrhagic fever. He found that the worker had, in fact, died of malaria.

Using a shot-gun sequencing approach, he was able to discover a new virus, Dandenong, in three transplant recipients who had died after receiving organs from the same donor.

He undertook a metagenomic survey of bees in which colony collapse disorder was observed. Through this, he was able to identify Israel Acute Paralysis Virus as the potential pathogen. A confirmation of the etiology was the ability of virus-specific RNAi to protect the bees from disease.

Studies of a child with X-linked agammaglobulinemia who had progressive encephalitis, lead to finding a virus that has 30% homology to mink astrovirus. The genome of this virus has been examined and it appears to be a new agent. Since two-thirds of all encephalitis is unexplained, this type of molecular diagnostic approach may help to resolve the etiology of these illnesses.

Dr. Lipkin indicated that he is also investigating a number of chronic diseases that are not though to be of infectious origin to determine if there infectious agents have a role in some of these disorders.

Challenges for the Development of Rapid, Point of Care Diagnostics
***Javan Esfandiari*, Executive Vice President, Chembio Diagnostic Systems, Inc.**

Dr. Esfandiari spoke about his company's experiences in developing a POC diagnostic for use in developing countries. He noted the importance of first determining whether a rapid assay is needed and state that the level of performance of the test may depend on the country of use. He indicated that the material you select as the test components (e.g., for the test strips) will be critical as one progresses in developing the diagnostic test and applying to different situations over time. He noted that there is more emphasis on developing quantitative assays. The shell life of a diagnostic may need to be longer than the usual 12 months for developing country use just because of the logistics of getting the product from the manufacturer to the site; sometimes 8-9 months are needed for this. The price that a country can pay per test may also determine the nature of the product that can be developed for in-country use.

He described how Chembio, which is a small public company, has made efforts over the last 5-6 years to move into the area of diagnostics for neglected diseases for use in developing countries. This has included developing a relationship with Inverness Medical Innovations as its US marketing partner and establishing a 10 year technology transfer agreement with the Brazilian government to make an HIV lateral flow test product. Chembio has also identified

other partners including the Centers for Disease Control, the Infectious Disease Research Institute, FIOCRUZ (Brazil), the Clinton Foundation and the US President's Emergency Plan for AIDS Relief. Dr. Esfandiari noted that Chembio has also benefited from funding from the NIH SBIR program.

Dr. Esfandiari provided several examples of challenges that have been encountered by Chembio. Their initial platform technology was a lateral flow test design. However, there were limitations in sensitivity and specificity associated with this design, particularly for more complex samples containing sputum, feces, urine. Chembio has now moved to a dual path platform (DDP™) to address some of these issues. This platform allows for independent flow paths for the sample and conjugate with the goal of increasing the analytical and clinical sensitivity. Multiple parameter tests are more feasible with the DDP™ technology.

Dr. Esfandiari described the ability to use a DDP™ rapid test for Leishmania as part of a public health program in Brazil which involves testing of dogs for Leishmania and killing those animals carrying the parasite so as to reduce one of the reservoirs of human disease. The previous approach had involved sampling and tagging the dogs and sending specimens to a laboratory. However, by the time the testing was done, it was very difficult to find the Leishmania-positive dogs. The rapid test used in the field avoids these logistical problems.

An example of another type of challenge was the request from a government for 1.5 million tests in the short time span of several months. The requesting country stated that they had received funds for implementing the diagnostic test, but the funds would be withdrawn unless the program was started in a fixed time frame.

A different type of challenge was related to the development of a reader for use with the visible bands that are the test result. Such a reader would facilitate the interpretation of weak bands and allow for the transfer of data to a computer. Chembio developed a hand-held DDP™ reader that would have cost $200 to $300 which is about ten-fold lower than most other readers. However, even that modest cost was considered to be "too high". There was also the complication related to the need to obtain batteries for the reader. In response to this problem, Chembio combined the DPP™ technology with fluorescence latex technology to provide a simple UV light for a sensitive dry chemistry fluorescence latex system for detection of pathogen without the need for using a reader.

Chembio is using the DPP™ technology to develop tests for antigens and antibodies for a number of infectious disease pathogens.

In response to questions, Dr. Esfandiari indicated that Chembio's manufacturing process is automated and that 3-5 people are need to manufacture the test strips in 3-4 days; while the manufacture of the test strips is relatively easy, the assembly of the kit is the difficult aspect of the production. In terms of antibody selection, they have determined that antibodies with affinities of less than 10^{-9} are not usable for a rapid system.

DISCUSSION and ADJOURNMENT

Dr. Lacourciere thanked all of the speakers for providing a better appreciation of diagnostic development. She reiterated the need for clear definition of intellectual property issues, obtaining advice from regulatory agencies, finding appropriate partners as well information on specific pathogen diagnostics and the development of point of care diagnostics.

平成20年度　日米医学協力計画「市民公開講座」

糖尿病・肝疾患・感染症の現状と課題　～日米医学協力計画の成果から～

◎ 日 時:

平成20年11月15日(土)

13:00～17:00 (開場　12:30～)

※日米医学関係者用の控え室は、

5Fの「5－A 会議室2」でございます。

◎ 会 場:

＜日本学術会議－講堂＞

地下鉄千代田線:乃木坂駅－出口5番

徒歩1分

◎ プログラム:

「市民公開講座」 糖尿病・肝疾患・感染症の現状と課題　～日米医学協力計画の成果から～				
時間	テーマ	演目内容	演者	
13:00-13:05	開会の辞			
13:05-13:15		挨拶	関係省庁の挨拶(予定)	
13:15-13:30		日米医学協力の概要	笹月　健彦	国立国際医療センター 名誉総長
13:30-13:55		日米医学の過去・現在・未来	アシュリー・ハース	ミネソタ州立大学 医学部微生物学: 教授
13:55-14:15	アジアの糖尿病	「日本人・アジア人糖尿病の特徴と予防のための生活の知恵」	清野　裕	関西電力病院: 院長
14:15-14:35	生活習慣病	「メタボリックシンドローム: なぜ太鼓腹がいけないの？」	川上　正舒	自治医科大学 附属さいたま 医療センター: センター長
14:35-14:45	質疑応答 (10分)			
14:45-14:55	休憩 (10分)			

14:55–15:15	生活習慣病と肝疾患	「アジアの肝臓が危ない―急増する生活習慣病NASH」	**西原 利治**	高知大学 医学部 消化器内科学：准教授
15:15–15:35	ウイルスと肝疾患	「C型肝炎から肝ガンに対する治療の進歩」	**泉 並木**	武蔵野赤十字 病院 消化器科： 副院長・部長
15:35–15:45	**質疑応答（10分）**			
15:45–16:05	新型インフルエンザ	「新型インフルエンザと次世代ワクチン」	**長谷川 秀樹**	国立感染症 研究所 感染病理部 第2室：室長
16:05–16:25	HIV感染症・エイズ	「エイズワクチン」	**山本 直樹**	国立感染症 研究所 エイズ 研究センター： センター長
16:25–16:45	結核	「結核とその世界の現状」	**石川 信克**	財団法人結核 予防会 結核 研究所：所長
16:45–16:55	**質疑応答（10分）**			
16:55–17:00	閉会の辞			

平成 20 年度日米医学協力計画　結核・ハンセン病専門部会会議プログラム

・日時：平成 21 年 2 月 27 日午後 1 時—2 月 28 日午前 12 時
・場所：(財)結核予防会結核研究所　4 階講堂
・一人質疑応答を含めて 20 分です。USB storage を準備してください。休憩は、取りませんので、各自、飲み物を、お取りください。5 時 30 分から 1 階食堂で懇親会を行います。1,000 円を徴収します。

＜2 月 27 日＞
12:50pm-1:00pm　開会挨拶と連絡事項

第1部　座長　菅原　勇
（1:00pm-2:20pm）
1．竹田潔「SLPI による結核感染制御」

2．菅原　勇「1 型および 2 型糖尿病と結核増悪について」

3．○吉村　満美子、仁木　誠、小林　和夫、松本　壮吉　「抗酸菌の休眠誘導と遺伝子発現解析」
4．○中島千絵、福島由華里、 Zeaur Rahim、鈴木定彦
「バングラデシュにおいてサルから分離された結核菌群菌の遺伝学的解析」

第 2 部　　座長　牧野正彦
(2:20pm-4:00pm)
5．田村敏生　「結核菌体ペプチドによる Th1 反応誘導機構の解析」

6．○和田崇之、長谷　篤「臨床由来結核菌株の集団構造と遺伝的多様性解析の試み」

7．岩本　朋忠　「わが国の結核菌集団構造のダイナミクス」

8．吉開泰信「マイコバクテリア感染に対する Th1 応答における T-T 細胞相互作用を介する CD30L/CD30 シグナルの新規の役割」

9．福富康夫　「クロファジミンのアポトーシス誘導機構：小胞体ストレス誘導並びに
細胞親和性に関わる因子の解明」

第 3 部　　座長　松岡　正典
(4:00pm-5:20pm)
10．後藤正道　「ミャンマーにおける純神経型ハンセン病の検討」

11．○後藤義孝, 小野寺澄子、福山紗千子、芳賀孟　「猫由来マクロファージ系株化細胞およびコットンラットマクロファージを用いた in vitro 抗酸菌感染系」

12．○宮本　友司、向井　徹、牧野　正彦　「Mycobacterium avium complex 由来 glycopeptidolipid の生合成解析」

13．松岡正典「薬剤耐性らい菌の簡易検出法の確立」

懇親会　5:30ｐm-7:00pm　1 階　食堂

＜2 月 28 日＞

第 4 部　座長　　光山　正雄
（8:30am-10:10am）
14．谷口初美　　「結核菌の細胞内増殖機構の解析における *M. smegmatis* J15CS－pYT923hyg の有

用性」

15. 杉田　昌彦　「ミコール酸含有糖脂質の生合成と免疫活性化」

16. ○河村伊久雄、　光山正雄「*Mycobacterium bovis* BCG 持続感染成立における PD-1/PD-L1 抑制性経路の関与」

17. ○向井　徹、前田百美、宮本友司、牧野正彦「抗酸菌感染症の予防に関する研究」

18. 松本　智成　「Anti-TNF agents for rheumatoid arthritis in patients with tuberculosis」

第５部　座長　吉開　泰信
(10:10am-11:50am)

19. 大原直也　「BCG thyX 欠損株の性状解析」

20. ○瀬戸慎太郎、小出幸夫　「結核菌ファゴソームから局在する Rab GTPase のファゴソーム熟成における機能解析」

21. 慶長　直人　「抗酸菌症と NRAMP-1 遺伝子多型の関連とその意義に関する検討」

22. 瀧井猛将　「ＢＣＧ亜株間のサイトカイン産生能の違いと加齢によるＢＣＧワクチンへの影響」

23. 岡田全司　　「新規結核ワクチンの開発と応用：HVJ/HSP65 DNA+IL-12 DNA ワクチンの治療効果」

11:50am-12:00pm　閉会と連絡事項　　牧野正彦

・パネル会議　12:00pm-1:0pm

社会保障国際協力推進研究推進事業
研究報告書

中国黒竜江省における多剤耐性結核に関する基礎的研究

研究代表者　菅原　勇　　（財）結核予防会結核研究所
共同研究者　Hong Ling　中国ハルピン医科大学微生物学教室
共同研究者　鈴木定彦　　北海道大学人獣共通感染症リサーチセンター

研究要旨
ハルピン医科大学付属病院で集められた結核菌臨床株１３５株を用いて、リンファンピシン、イソニアジド、ストレプトマイシン、エタンブトール耐性を調べた（比率法）。リファンピシンのみの耐性株は 4 株、リファンピシンと他の結核薬 1 剤耐性株は、13 株、リファンピシンと他の結核薬 2 剤耐性株は、27 株、リファンピシンと他の結核薬 3 剤耐性株は、44 株存在した。即ち、多剤耐性結核が多かった。多剤耐性結核の定義に当てはめると、リファンピシンとイソニアジドの両方に耐性である結核菌株は、39 株存在した。サンプル数は、少ないが、黒竜江省では、多剤耐性結核が多いと推定される。

A. 研究目的
昨年、上海市肺科医院における、多剤耐性結核の実情を調べた。この研究で、わかったことは、多剤耐性結核の実情を調べるためには、地方都市での結核発生率を調べることが重要と痛感した。今般、ハルピン医科大学 Hong Ling 教授の支援を得て、黒竜江省における多剤耐性結核の実情を調べることにした。

B. 研究方法
ハルピン医科大学付属病院で集められた 135 の臨床株を使用した。固形培地に、リファンピシン（RFP）、イソニアジド(INH)、ストレプトマイシン(SM)、エタンブトール（EMB）を加えて、一定に希釈した結核菌臨床株を加えた。増殖率を、薬剤を加えないプレートと比較して薬剤耐性度を調べた（比率法）。今回は、最初の実験だったので、別の検査（MIC plate を用いた方法）と結核菌 DNA を用いた標的遺伝子の突然変異解析は行わなかった。

C. 研究結果
実験結果は、表 1 にまとめてある。多剤耐性結核の中心薬剤である RFP, INH に着目する。リファンピシンのみの耐性株は 4 株、リファンピシンと他の結核薬 1 剤耐性株は、13 株、リファンピシンと他の結核薬 2 剤耐性株は、27 株、リファンピシンと他の結核薬 3 剤耐性株は、44 株存在した。即ち、多剤耐性結核が多かった。多剤耐性結核の定義に当てはめると、リファンピシンとイソニアジドの両方に耐性である結核菌株は、39 株（30%）存在した。サンプル数は、少ないが、黒竜江省では、多剤耐性結核が多かった。

D. 考察
サンプル数（135 株）は少ないが、多剤耐性結核が多いと推察された。今回、薬剤を 4 種類に絞ったが、多剤耐性結核が多いと考えられる。従って、XDR-TB も当然多いと予想される。次回、カナマイシン、カプレオマイシン、レボフロキサシン、アミカシンを用いて、これらの薬剤耐性を調べ、XDR-TB の頻度を調べたい。

また、これら結核菌を用いて DNA を抽出し、薬剤標的遺伝子の突然変異を調べ、どの変異パターンが多いかを調べることも重要である。次回の実験で、検討したい。

黒竜江省 CDC の担当者に、黒竜江省の年間新規結核発生数を尋ねたが、正確な統計は存在しない。結核患者登録システムは、まだ確立されていない。

黒竜江省での結核患者の有意な減少を目指すには、結核を研究する研究者が少ない。インフルの整備が急務である。機会があれば、若手研究者を日本で訓練して、黒竜江省で結核対策に取り組めるようにしたい。

E. 結論
サンプル数は、少ないが、黒竜江省では、多剤耐性結核が多いと推定された。

F. 健康危険情報
なし

G. 論文発表

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2．学会発表　なし

H. 知的財産権の出願・登録状況

1．特許取得　なし

2．実用新案登録　なし

3．その他

論　　文　　集