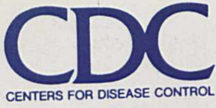

Applications of Biotechnology for Infectious Diseases

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U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control

Center for Infectious Diseases

and

CDCs Technology Transfer Office

INTRODUCTION

"It is characteristic of science and progress that they continually open new fields to our vision." Louis Pasteur, the famous French microbiologist, spoke those words more than a hundred years ago, during the first "Golden Age" of microbiology. At that time, through the introduction of culture media, sterile technique, and new staining methodologies, Pasteur, Koch and others, proved that microorganisms caused many of the devastating diseases that had ravaged civilization over the millenia. Within a few short years of their discoveries, centuries-old traditions and beliefs, which held that diseases were transmitted by some peculiar miasmas, quickly fell into disfavor. All of the possible applications of the new technology were not apparent at the time, but many of the more far-sighted individuals, Pasteur among them, realized that they were at a watershed moment in human history. The incredible improvements in the health and life span of humans over the last hundred years attest to their vision.

Contemporary visionaries contend that we are currently poised at another great moment in medical history. The identification of DNA as the hereditary material and the deciphering of the genetic code provided the prologue for the current Golden Age. The subsequent development of new technologies, which allow scientists to identify, characterize, and manipulate specific genes and to track the distribution and movement of other molecules within cells, provide the tools that will be used to complete the revolution. And although it is difficult to predict the specific outcome of these current scientific and technologic breakthroughs, one result does seem certain - betterment of the human condition.

CDC is actively engaged in the practical applications of this explosion of new technology to disease problems through a Congressional mandate. Until 1986, the principal methods used by the Centers for Disease Control (CDC) to transfer technology outside the Government were training, education, and information dissemination. In 1986, Congress passed the Federal Technology Transfer Act of 1986 to improve the link between the Federal laboratories' technology base and U.S. businesses. This law and subsequent Executive Order 12591 (April 10, 1987) authorize Federal laboratories to patent and exclusively license inventions to and collaborate with businesses on research and development.

The enclosed abstracts address the research in the application of increasingly complex technology to diagnostics. The application of basic research leads to improved diagnostic tools which in turn enhances epidemiology.

If you would like additional information on the above or information on CDC's cooperative research policies and procedures, please contact either:

Dr. Joseph McDade
Associate Director for
Laboratory Science
Center for Infectious Diseases
Centers for Disease Control
1600 Clifton Road; Mailstop C12
Atlanta, GA 30333
(404) 639-3967

or

R. Eric Greene
Technology Transfer Coordinator
Centers for Disease Control
1600 Clifton Road; Mailstop A20
Atlanta, GA 30333
(404)639-3812

CDC INFORMATION CENTER
CENTERS FOR DISEASE CONTROL
ATLANTA, GA 30333

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ADVANCES IN VACCINE DEVELOPMENT FOR ENCAPSULATED BACTERIA.

Claire V. Broome, Jay Wenger, Balasubr Swaminathan, and George M. Carlone, Meningitis and Special Pathogens Branch, Division of Bacterial Diseases

Serum antibodies directed against the polysaccharide capsule of bacteria responsible for meningitis and sepsis are capable of protecting against human disease. However, infants who are at highest risk of disease are unable to respond to polysaccharide antigens. Recent research has shown that covalently linking the polysaccharide to a protein carrier is effective in eliciting antibody in infants. Trials are currently under way to evaluate clinical protection induced by four different *Haemophilus influenza* type b conjugate vaccines. Our work focuses on the performance of conjugate vaccines, variables that might affect immunogenicity in different conjugates, and potential applications for this technology in development of vaccines for other high priority diseases such as *Neisseria Meningitidis*, group B streptococcus, and the pneumococcus.

BACTERIAL ISOPRENOID QUINONE STRUCTURE DETERMINATION.

George M. Carlone and Susan H. Turner, Meningitis and Special Pathogens Branch, Division of Bacterial Diseases

The chemical structure of novel class of methyl-substituted menaquinones (2,[5 or 8]-dimethyl-3-farnesyl-farnesyl-1,4-naphthoquinone) was determined using ultraviolet and mass spectroscopy and nuclear magnetic resonance analysis. Quinones are useful markers for taxonomic and epidemiologic groupings since they are widely distributed, structurally diverse, and physiologically significant. Isoprenoid quinones of bacteria are lipophilic components of the cytoplasmic membrane that play important roles in the transfer of electrons in the respiratory chain and function in oxidative phosphorylation and active transport. Determination of the quinone composition in the electron transport chain defines the membrane-associated energy transduction potential of a given bacterial species. The class of quinone and the length and degree of saturation on the isoprenoid side chain are useful in bacterial taxonomy.

BACULOVIRUS-EXPRESSED ANTIGENS FOR HERPESVIRUS SERODIAGNOSTIC TESTS.

Demetrio Sanchez-Martinez and Philip E. Pellett, Viral Exanthems and Herpesvirus Branch, Division of Viral and Rickettsial Diseases

Herpes simplex virus type 1 (HSV-1) glycoprotein G (gG-1) and its HSV-2 counterpart (gG-2) are type-specific antigens that allow serologic discrimination of prior infection with HSV-1 from prior infection with HSV-2. Epstein-Barr virus (EBV) specifies a glycoprotein, gp110, which is the basis of a sensitive EBV-specific serologic test. The utility of assays based on these proteins has been limited by the difficulty of preparing large amounts of the purified antigens. We have expressed these antigens in a baculovirus gene expression system using a novel transfer vector that mimics the nucleotide sequence of the wild-type baculovirus polyhedrin gene 5' nontranslated region, and allows construction of recombinant viruses differing from the wild-type baculovirus only in the coding sequence of the gene to be expressed. The level of expression using this vector is higher than that obtained using a vector containing extraneous nucleotides in the polyhedrin 5' non-coding sequence. The baculovirus-expressed proteins are recognized by human serum specimens, the reactions with gG-1 and gG-2 being HSV type-specific.

CD4 AND HIV: ANALYSIS OF BINDING SITE INTERACTIONS AND POTENTIAL FOR THERAPEUTICS.

J. Steven McDougal, Alison Mawle, Janet Nicholson, Thomas Hodge, Sherry Orloff, and M. Susan Kennedy, Immunology Branch, Division of Immunologic, Oncologic, and Hematologic Diseases

CD4 is a cell surface protein found at highest density on a subset of T lymphocytes and at lower density on other hematopoietic cells such as macrophages. CD4 T cells perform critical antigen recognition and helper/inducer functions. CD4 has also been shown to function as a receptor for HIV-1. The CD4 gene was first cloned and sequenced by Maddon et al. In collaboration with this group, we have been involved in genetic manipulations of CD4 and its expression in various cellular environments. In one such manipulation, introduction of a stop codon at the extracellular-transmembrane junction of CD4 cDNA and expression of the construct in mammalian cells resulted in a secreted form of soluble CD4 (sCD4). sCD4 binds to HIV-1 envelope glycoprotein, blocks HIV-1 binding to CD4 T cells, and effectively inhibits HIV-1 infectivity for T cells and macrophages in vitro. sCD4 is now in phase I/II clinical trials. Physicochemical studies and mapping studies with truncated and mutagenized forms of sCD4 have further localized the binding site of HIV-1 and its structural constraints. Such studies may facilitate the design of more effective (second generation) constructs of CD4 that prolong half-life, increase valence, or change affinity, or that deliver toxins, drugs, or mediator molecules to virus-infected cells.

DETECTION AND CHARACTERIZATION OF HEPATITIS A VIRUS (AND OTHER FASTIDIOUS VIRUSES) BY IMMUNOSELECTION AND ENZYMATIC AMPLIFICATION OF NUCLEIC ACID.

Betty Robertson, Omana Nainan, Bhaiona Khanna, Vicki K. Brown, and Harold S. Margolis, Hepatitis Branch, Division of Viral and Rickettsial Diseases

Polymerase chain reaction (PCR) amplification of nucleic acid present in clinical, environmental, or fomite-derived samples is a highly sensitive method for detection of infectious viral agents that cannot be identified by traditional cell culture approaches. However, the inherent character of these types of samples introduces a multitude of potential problems which complicate amplification and analysis. Using hepatitis A virus (HAV) as a model, we have approached the detection and identification of wild-type viruses in clinical specimens by an initial antibody capture of virus prior to PCR amplification. Immunocapture of virus with specific antibody followed by reverse transcription, PCR amplification, and traditional hybridization techniques results in the detection of between 30 and 300 infectious viral particles, while the use of a 5' end-labeled primer during the PCR reaction increases sensitivity of detection by approximately one log. The primer pair used for amplification was selected to bind relatively conserved sequences which flank a variable region within the VP1 coat protein, and has resulted in the amplification and detection of HAV agents that differ genetically by 20-25%. The use of these approaches can be applied to detect virus within potentially infected foods or other environmental sources of HAV contamination.

DEVELOPMENT AND EVALUATION OF ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR DETECTION OF SHIGA-LIKE TOXIN I AND SHIGA-LIKE TOXIN II.

Frances P. Downes, James H. Green, Katherine Greene, Nancy Strockbine, Joy G. Wells, and I. Kaye Wachsmuth, Enteric Diseases Branch, Division of Bacterial Diseases

Shiga-like toxin (SLT)-producing *Escherichia coli* have been associated with a spectrum of human illnesses, including hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP), as well as with edema disease in swine. Two antigenically distinct groups of toxins designated SLT-I and SLT-II have been identified. We have developed two sandwich enzyme-linked immunosorbent assays (ELISAs) based on two toxin-specific antibodies: a monoclonal capture antibody and a rabbit polyclonal second antibody. When compared with the conventional cytotoxicity neutralization assay, the ELISAs were 100% sensitive and specific for identifying moderate to high-level SLT-producing *E. coli* among 166 human and 54 animal isolates tested. Early diagnosis of SLT-producing *E. coli* infections is important in controlling person-to-person transmission in outbreak situations and in monitoring infected individuals for development of HUS or TTP. The SLT ELISAs provide an attractive alternative to cell culture for identifying these organisms and should facilitate epidemiologic studies of SLT-producing *E. coli* associated with human and animal infections.

DIAGNOSIS OF NEW HUMAN RETROVIRUSES.

Tom M. Folks, Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases

Detection and isolation of new and unknown human retroviruses from clinical material will depend on an understanding of the target cell type in which the agent replicates. Modification of the cell may be needed to make it permissive to virus infection and replication in vitro. Currently we are developing methods for cytokine and cell membrane perturbation which "activate" retroviruses from latent states. Following cellular activation, culture supernatants containing putative retroviruses are concentrated, solubilized, and reverse transcribed. The resultant cDNA is amplified by polymerase chain reaction (PCR) using "consensus" primers from known mammalian retroviral genes (LTR, GAG, POL). The amplified products are then directly sequenced for known retroviral sequences or used as molecular probes. The combined technologies of recombinant cytokine cellular modification and PCR should provide new capabilities for identifying retroviral agents from diseases of unknown etiology.

EXPRESSION OF INFLUENZA A AND B NUCLEOPROTEIN ANTIGENS IN BACULOVIRUS.

Paul A. Rota, Renee A. Black, Barun K. De, and Alan P. Kendal, Influenza Branch, Division of Viral and Rickettsial Diseases

Full-length cDNA clones of the nucleoprotein (NP) genes of influenza A/Ann Arbor/6/60 and B/Ann Arbor/1/86 viruses were constructed from virion RNA and subsequently expressed in *Spodoptera frugiperda* (Sf9) cells using the baculovirus vector, *Autographa californica* nuclear polyhedrosis virus (AcNPV). Western blot analysis of lysates prepared from Sf9 cells infected with the recombinant viruses confirmed that the baculovirus-expressed NP antigens were reactive with monoclonal antibodies specific for either type A or B NP and with anti-NP antibodies in human serum samples. Electrophoretic analysis indicated that the expressed influenza NP antigens comigrated with NP purified from influenza A or B virions and that the recombinant NP antigens represented greater than 10% of total protein in infected cells. Dilutions of clarified Sf9 cell lysates were used as antigens in a standard enzyme immunoassay format to detect serum antibody specific for influenza A or B viruses. The results from assays using the baculovirus-expressed NP antigens showed close correlation with the results obtained using bacterially-expressed NP antigen as well as complement fixation. Therefore, baculovirus-expressed NP antigens could potentially be used to develop a standardized assay system for the serodiagnosis of influenza virus infections as a more convenient alternative to existing methods.

GENERATION, IDENTIFICATION, AND ISOLATION OF BISPECIFIC MONOCLONAL ANTIBODIES.

Edwin W. Ades, Carolyn Dawson, Janet Nicholson*, and J. Todd Parker, Biological Products Branch, Scientific Resources Program, and Immunology Branch, Division of Immunologic, Oncologic, and Hematologic Diseases**

Enhanced tumor targeting, by cross-linking effector to target cells, has been previously demonstrated by several investigators using bispecific antibodies. Hybrid-hybridomas are selected via two-color intracellular fluorescent dyes (rhodamine 123 and dihydroethidium), isolation of the two-color fusion population is accomplished by fluorescence-activated cell sorting (FACS), and analysis of the fusion efficiency is performed. Antibody targeting specificity is examined using FACScan analysis of the respective treated target cells. The lytic efficacy of our bispecific antibody (anti-melanoma x anti-CD3) is examined both in vitro and in vivo following treatment with bispecific antibody. A higher melanoma lytic response following treatment with our bispecific antibody, in comparison with treatment with either parental monoclonal antibody, has been demonstrated. This provides new opportunities for adoptive immunotherapy and identifying new anti-proliferative activities for these antibodies.

GENETIC ENGINEERING IN MAMMALIAN CELLS: VEHICLES FOR GENE TRANSFER.

Edwin W. Ades, Francisco Candal, and Debra Musgrove, Biological Products Branch, Scientific Resources Program

Mammalian cells will express exogenously introduced genes provided that the genes possess the proper DNA regulatory elements recognized and used by the host cell. The yield of protein product from an introduced gene can be increased by several means; one is to increase the actual number of DNA copies of the gene within each cell (gene amplification), another is to use control sequences that may respond to external signals. Our goal is to introduce a gene into a mammalian cell under the control of an inducible promoter. Large scale growth of mammalian cells that require long-term cell viability prior to external signal has been established as well as protein products. These cells may be a suitable cellular vehicle for the introduction and control of therapeutic genes into patients.

HUMAN-RODENT HYBRID CELLS AS A MODEL FOR FACTORS THAT REGULATE HUMAN RETROVIRUS EXPRESSION.

Clyde Hart, Judith Galphin, Chin-Yih Ou, and Gerald Schochetman, Laboratory Investigations Branch, Division of HIV/AIDS

Human retroviruses contain nonstructural regulatory genes that control viral expression in an infected host cell. In many instances these regulatory genes are believed to work in concert with or induce expression of host cell factors to effect virus production and pathogenesis. To investigate the human-specific host cell factors involved in regulating expression of the retrovirus HIV, human-Chinese hamster ovary hybrid cells with defined sets of human chromosomes were assayed by DNA transfection. High levels of virus production and viral trans-activator gene-induced activation of the viral long terminal repeat (LTR) was present only in cells containing human chromosome 12. High levels of trans-activation of the LTR from HTLV I and II retroviruses, however, was not supported by hybrid cells containing human chromosome 12. This indicates that the regulatory genes of diverse human retroviruses require different host cell factors to support elevated gene expression. Identification of specific host cell factors involved in the retroviral disease process may provide new targets for the development of antiviral therapy.

HUMAN IMMUNE RESPONSES TO *ONCHOCERCA VOLVULUS*: POSSIBLE RELATIONSHIP BETWEEN SPECIFIC ISOTYPIC REACTIVITY TO ANTIGENS AND STATE OF IMMUNITY.

Ann E. Boyer, Victor C. Tsang, Mark L. Eberhard, Joy A. Brand, Wei Zhou, and Laureen Laughnan, Parasitic Diseases Branch, Division of Parasitic Diseases

Onchocerciasis, a leading cause of human blindness, affects an estimated 20-50 million persons. Isotypic differences in immune responses to *Onchocerca volvulus* antigens were assessed for 798 persons residing in endemic-disease Guatemalan areas by falcon assay screening test enzyme-linked immunosorbent assay (FAST-ELISA) and immunoblot (western blot). The population was separated into four groups based on clinical status: N+/-F+, N+F-, N-F-H+, and N-F-H-, where N = nodule (adult *O. volvulus*), F = microfilaria, and H = history. Isotope quantification by FAST-ELISA showed that IgG1 reactivity to *O. volvulus*-specific antigens was predominant in the majority of infected persons and was from 3 to 5 times lower in putatively "immune" persons. Immunoblots showed frequent reactivity to a group of glycoproteins at 20 kilodaltons by IgG3 in most uninfected persons from hyperendemic-disease areas and in some N+F- persons. Conversely, in most F+ persons, reactivity to this antigen was by IgG1 and not by IgG3. On immunoblot, a mangabey inoculated with L3s of *O. volvulus* began to recognize GP20 at 2 weeks post-inoculation. At this early date, antigen recognition was presumably elicited by the L3 stage , suggesting that GP20 may be a common adult/larval stage antigen. The fact that GP20 is predominantly recognized by IgG3 in uninfected persons and some amicrofilaremic persons suggests that IgG3 activity to GP20 may be associated with acquired immunity and offers a potential target antigen for vaccine development.

HYBRIDIZATION PROTECTION ASSAY FOR RAPID AND SENSITIVE DETECTION OF NUCLEIC ACIDS.

Chin-Yih Ou, Laboratory Investigations Branch, Division of HIV/AIDS

Nucleic acid amplification via polymerase chain reaction (PCR) is widely used in the detection and quantification of genetic information in various genetic defects and infectious agents including human immunodeficiency virus. Current methods for the detection of the amplified DNA are time-consuming, labor-intensive, and often involve radioisotopic oligonucleotide probes. To overcome these drawbacks, we have applied a hybridization protection assay (HPA) using chemiluminescent acridinium ester (AE)-labeled, single-stranded oligonucleotide probes. The AE groups on the unhybridized probe are highly sensitive to alkaline hydrolysis but are resistant when the probe is hybridized with the amplified DNA. Thus simple alkaline hydrolysis can selectively remove the AE on unhybridized probe in the hybridization solution without physical separation as required for other detection methods such as gel electrophoresis and blotting. The quantity of the remaining AE on the hybridized probe is measured by a luminometer. The sensitivity of HPA is comparable to that of the 32P-labeled probes. The assay offers a rapid (1 hour), quantitative, and highly reproducible measure of PCR-amplified DNA.

IDENTIFICATION OF EPITOPES IN THE P24 (GLYCOSAMINOGLYCAN) PROTEIN OF HIV-1 THAT ELICIT A CYTOTOXIC T LYMPHOCYTE RESPONSE IN INFECTED PERSONS.

Alison C. Mawle and J. Steven McDougal, Immunology Branch, Division of Hematologic, Oncologic, and Immunologic Diseases

We have studied the cytotoxic T lymphocyte (CTL) response to the p24 (glycosaminoglycan) antigen in long-term survivors from a San Francisco cohort of persons who have been HIV-1 seropositive and completely asymptomatic for an average of 9 years. This group was compared with a group of AIDS patients from the same cohort who had also been infected for an average of 9 years. The assay was performed using freshly isolated peripheral blood lymphocytes (PBL) as effector cells, and autologous Epstein-Barr virus-B (EBV-B) cells infected with a p24 vaccinia construct were used as target cells. We have shown that persons who are asymptomatic have a CTL response to p24 that is major histocompatibility complex-restricted, whereas persons with AIDS have no response to p24.

We have a panel of nine peptides derived from p24 that are potential T cell epitopes, and we are using these to determine important epitopes in this response. Freshly isolated PBL will be assayed for CTL activity on autologous EBV-B cells pulsed with each peptide. We hope to identify a series of peptides that will act as CTL targets in conjunction with the major HLA determinants in the population, and which therefore could be used as the basis of a vaccine. We plan to try various techniques using these peptides to stimulate CTL in both mouse and primate models.

IDENTIFYING DIVERSE MICROBES WITH CERTAIN WELL-CONSERVED GENES: POLYMERASE CHAIN REACTION/RESTRICTION FRAGMENT LENGTH POLYMORPHISM AS APPLIED TO RICKETTSIAE.

Russell Regnery, Catherine Spruill, M. Drancourt, and Brian D. Plikaytis, Division of Viral and Rickettsial Diseases, Statistical Services Activity, Viral and Rickettsial Zoonoses Branch, Division of Bacterial Diseases

We have developed a novel, simple, and fast method for differentiation of various species and genotypes of rickettsiae. Polymerase chain reaction amplification of specific gene(s), using well conserved "universal" primer sequences, was used to produce DNA products from all rickettsiae tested. This amplified DNA was subjected to restriction-endonuclease digestion and analysis on polyacrylamide gels. These digests produced diagnostic, species-specific patterns; isolates from clinical samples were readily identified in this manner. Divergence between the genes of various species can also be estimated. These methods should be applicable to a wide variety of bacteria.

ISOLATION AND PURIFICATION OF A SPECIES-SPECIFIC *STREPTOCOCCUS PNEUMONIAE* PROTEIN ANTIGEN BY ISOELECTRIC FOCUSING.

Harold Russell and Jean A. Tharpe, Respiratory Disease Branch, Division of Bacterial Diseases

Streptococcus pneumoniae is a leading cause of morbidity and mortality in the United States and developing countries. Except for detection of antigen in cerebrospinal fluid, existing antigen and antibody tests have not proved to be diagnostically useful because of low sensitivity and specificity. A new species-specific protein isolated from *S. pneumoniae* has been purified to electrophoretic homogeneity by isoelectric focusing. The protein has a molecular mass of 37 kilodaltons (kDa) and a pI of 5.2. Monoclonal antibodies to the 37-kDa antigen do not react with 62 other strains representing 15 genera that may also cause lower respiratory diseases. The monoclonal antibodies reacted with all capsular types of *S. pneumoniae* tested (N = 21). The effectiveness of the purified 37-kDa antigen as a useful immunodiagnostic marker for the etiologic diagnosis of infection is under study.

MAINTENANCE OF VIRAL LATENCY AND REGULATION OF VIRAL GENE EXPRESSION: DETECTION OF VIRUS BY IN VIVO FOOTPRINTING.

Daniel P. Bednarik, Patricia C. Guenthner, Nora J. Besansky, and Tom M. Folks, Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases

Infection of cells by HIV can result in a period of quiescence or latency, which may be obviated by treatment with various inducing agents such as 5-azacytidine. Evidence from our experiments suggests that several CpG sites exist to form a "CpG island" within the HIV core enhancer, and block the expression of viral mRNA when these sites are methylated. Both viral and cellular *trans*-acting factors are able to overcome this transcriptional block. In addition, such changes in the methylation profile of the long terminal repeat (LTR) can alter the interaction of DNA: protein binding, and a new class of binding proteins which recognize the methylated HIV LTR is suspected. In these studies, we are developing a direct method of genomic sequencing and in vivo footprinting to elucidate the nature of these sites in both latent and productive viral states. This technique may be eventually exploited to detect viral genomic sequences directly from primary viral isolates.

MAPPING SURFACE-EXPOSED EPITOPES OF THE *CHLAMYDIA TRACHOMATIS* MAJOR OUTER MEMBRANE PROTEIN.

Jim Newhall, Molecular Epidemiology and Pathogenesis Branch, Division of Sexually Transmitted Diseases Laboratory Research

The major outer membrane protein (MOMP) of *Chlamydia trachomatis* functions both as a structural protein and as a channel-forming protein through the outer membrane. In addition, this protein accounts for over 60% of the outer membrane protein mass and is the principal antigenic component at the surface of the organism. As such, MOMP represents the main target for potentially neutralizing host immune responses. Thus, there is considerable interest in MOMP for the development of vaccines to prevent chlamydial disease including trachoma as well as sexually transmitted diseases. The purpose of our current studies is to identify the amino acid sequences of MOMP that make up some of the epitopes of this protein that are known to give rise to neutralizing antibody. Three separate methods have been used to localize MOMP epitopes. In the first, the protein is digested briefly with a protease (V8 or chymotrypsin) that cuts the protein into fairly large fragments (>10 kilodaltons). The location of different epitopes on individual fragments can then be determined by immunoblotting. The second method of mapping utilizes synthetic hexapeptides that collectively span known antigenic regions of the protein. By screening overlapping peptides, it is possible to locate the primary binding sequence of epitope-specific monoclonal antibodies. The third mapping technique uses plasmid expression vectors to create hybrid proteins consisting of β-galactosidase and any polypeptide sequences desired. Using these methods we have been able to determine the amino acid sequences that make up five different surface-exposed epitopes of the chlamydial MOMP. These sequences are currently being used to construct immunogens for vaccine testing in animal models.

NEWER APPROACHES TO IDENTIFICATION OF RESPIRATORY VIRUSES: CULTURES, DIRECT DETECTION, PROBES.

John C. Hierholzer, Respiratory and Enterovirus Branch, Division of Viral and Rickettsial Diseases

The 1990s have opened with challenging opportunities in the field of viral diagnosis, especially rapid viral diagnosis. Changes in our approaches to respiratory virus identification are being brought about by problems with monkeys and monkey-derived tissue culture, by the expenses incurred in lengthy culturing and routine identification tests, and by genetically intermediate strains which defy straightforward identification. For example, herpes B, Ebola, and simian immunodeficiency viruses are adventitious monkey agents that are making the use of tissue products from monkeys both unsafe and impractical. As expenses with cell cultures and media components multiply, we are substituting enzyme- and time-resolved fluoroimmunoassays in rapid diagnostic formats to circumvent the need for extensive culturing. To identify respiratory viruses by their genetic properties, we are developing specific DNA primer pairs and DNA probes; genomic sequence analyses of field isolates are under way to assist in the refinement of molecular reagents of high specificity and sensitivity. Our goals are to employ and develop rapid diagnostic techniques to identify whole virus, viral antigens, or viral nucleic acid directly in clinical specimens, to culture only the positive specimens and to minimize culturing to only what is needed for preserving the strains for future use.

RABIES DIAGNOSTIC REAGENTS PREPARED FROM A RABIES N GENE RECOMBINANT EXPRESSED IN BACULOVIRUS.

Frances Reid-Sanden, John W. Sumner, Jean S. Smith, MaKonnen Fekadu, John H. Shaddock, and William J. Bellini, Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases

A gene encoding the nucleoprotein (N) of rabies virus was inserted into the genome of the baculovirus *Autographa californica* nuclear polyhedrosis virus. Recombinant gene expression was controlled by the polyhedrin gene promoter. Insect cells (*Spodoptera frugiperda*) infected by a baculovirus recombinant containing the N gene insert produced a novel 55-kilodalton protein comparable in size to the rabies N protein, as demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This new gene product retained the antigenic and immunogenic properties of the native viral protein in its ability to react in immunoprecipitation and immunofluorescence assays with antirabies antibodies. The baculovirus expression system provides a safe, convenient, and inexpensive source of N protein for the production of antiserum and adsorbing suspensions for use in rabies diagnoses.

RAPID CONFIRMATION OF *LISTERIA MONOCYTOGENES* BY A NONISOTOPIC DNA COLONY HYBRIDIZATION METHOD.

Balasubr Swaminathan, Meningitis and Special Pathogens Branch, Division of Bacterial Diseases

An oligonucleotide probe was derived from the sequence of a 321-base pair internal fragment of *msp* gene encoding a major secreted polypeptide of *Listeria monocytogenes*. The probe was labeled with digoxigenin using terminal deoxynucleotidyl transferase, and its specificity was determined by dot-blot assays. The probe reacted with all strains of *L. monocytogenes* tested (12 of 12 strains representing 5 serotypes). It did not react with any other *Listeria* species or with other gram-positive bacteria tested (*Brochothrix, Erysipelothrix, Corynebacterium, Rhodococcus, Lactobacillus, Leuconostoc, Bacillus, Staphylococcus,* and *Streptococcus*). The nonisotopic DNA colony hybridization assay was used for the rapid confirmation of *L. monocytogenes* on *Listeria*-selective agars that had been streaked with food enrichment cultures. Sixty-six food samples were tested by conventional confirmation methods and the DNA colony hybridization assay. The sensitivity and specificity of the colony hybridization assay were 100% and 97%, respectively. The DNA colony hybridization assay will be useful for quality control of processed foods, particularly semi-perishable foods with limited shelf-life.

ROLE OF A 60-MDa PLASMID IN THE PATHOGENESIS OF *ESCHERICHIA COLI* 0157:H7 DIARRHEA.

Timothy J. Barrett, Istvan Toth, James H. Green, Mitchell L. Cohen, Hella S. Rumschlag, and I. Kaye Wachsmuth, Enteric Diseases Branch, Division of Bacterial Diseases

Escherichia coli strains of serotype 0157:H7 can cause a severe diarrheal illness (hemorrhagic colitis) in humans which often results in serious sequelae. These strains typically produce Shiga-like toxins and cause attaching and effacing lesions in the colons of animal models. Virtually all isolates contain a 60-MDa plasmid which does not code for toxin production. We investigated the role of this plasmid in the pathogenesis of hemorrhagic colitis. By comparing a plasmid-cured strain with a wild-type 0157, we found that the plasmid aided in bacterial adherence. *E. coli* HB101, transformed with the 60-MDa plasmid, caused attaching and effacing lesions in human intestinal cell cultures. Electron micrographs revealed electron dense areas in the intestinal cells adjacent to bacterial cells; this may represent actin polymerization at the site of attachment. The 60-MDa plasmid also mediated the production of two proteins identified by enzyme-linked immunosorbent assay (ELISA) and Western blot analysis based on absorbed polyclonal antisera. These proteins were found in all *E. coli* 0157:H7 strains and in other serotypes of Shiga-like toxin-producing *E. coli*; traditional EPEC serotypes were also ELISA positive. One or both of these proteins may represent a newly recognized virulence factor common to several important enteric pathogens. This ELISA should thus be valuable in identifying both pathogenic *E. coli* and potentially pathogenic *E. coli* that are currently unrecognized by traditional assays. These proteins may also serve as a basis for future vaccine development.

SEROLOGIC DISCRIMINATION OF HTLV I AND HTLV II BY USING AN IMMUNODOMINANT EPITOPE OF HUMAN T-LYMPHOTROPHIC VIRUS TYPE I.

Renu Lal, Donna Rudolph, Michael Lairmore, Rima Khabbaz, John Coligan, and Tom Folks, Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases

A series of synthetic peptides derived from the *gag*, *pol*, and *env* genes of HTLV I and II was selected based upon differences in amino acid sequences and predicted secondary structure. A total of 87 serum specimens from HTLV I- and HTLV II-infected persons were used to test for the specificity of the assay. Two peptides (P_3,E_2) reacted with both HTLV I- and HTLV II-infected sera (80-90%); the other two peptides (G_{1a},E_1) reacted with 90% of HTLV I-infected sera and 9-11% of those with HTLV II infection. In contrast, E_5 a peptide derived from envelope region of HTLV I reacted with all HTLV I-positive sera and none of the HTLV II sera. Further characterization of E_5 epitope demonstrated that it represents a native epitope(s) exposed on the HTLV I virus-infected cells. We therefore conclude that the E_5 peptide represents an immunodominant domain of HTLV I and that E_5-based assays distinguish between infection by these closely related viruses.

USE OF 16S rRNA GENE SEQUENCES IN THE DETECTION OF *CHLAMYDIA* SPECIES BY POLYMERASE CHAIN REACTION.

Patricia Fields and Peter Berdal, Division of Sexually Transmitted Diseases Laboratory Research and Division of Bacterial Diseases

16S rRNA gene sequences have been used extensively to assess evolutionary relatedness among bacteria because these genes are present and serve a similar function in all bacteria, and they exhibit both highly conserved and variable sequences. The variable regions from these genes have been used as the target for DNA probes, and more recently for polymerase chain reaction (PCR) assays that specifically identify or detect groups of organisms. We have used sequences from the *Chlamydia trachomatis* and *C. pneumoniae* 16S rRNA genes as primers in PCR assays that are specific for each of these species. The *C. pneumoniae* primers do not recognize *C. trachomatis* DNA; the *C. trachomatis* primers do not recognize *C. pneumoniae* DNA. Additionally, neither primer pair detected any other bacterial species tested. We have used the PCR assays to detect DNA from one to 10 laboratory grown organisms, and to detect chlamydial DNA in clinical specimens. The success that we have had using 16S r RNA genes as the basis for PCR tests for *Chlamydia* spp. suggests that 16S rRNA genes may be a good starting point in the development of PCR assays for other bacterial pathogens.

Centers for Disease Control and
Agency for Toxic Substances and Disease Registry

POLICY STATEMENT ON COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS AND INTELLECTUAL PROPERTY LICENSING

This Statement sets forth the policies of the Centers for Disease Control (CDC) and the Agency for Toxic Substances and Disease Registry (ATSDR) on various aspects of cooperative research and intellectual property licensing. These policies apply to the negotiation of CDC and ATSDR Cooperative Research and Development Agreements (CRADAs). License agreements for intellectual property rights to inventions developed under a CRADA or through the CDC and ATSDR intramural research programs whether negotiated by CDC and ATSDR or by the National Technical Information Service on its behalf, will also incorporate these policies. This statement may be revised as appropriate.*

The Federal Technology Transfer Act of 1986, (FTTA, 15 U.S.C. at Section 371O), Executive Order 12591 of April 10, 1987 orders Federal laboratories to assist universities and the private sector in broadening our national technology base by moving new knowledge from the research laboratory into the development of new products and processes. While Federal patent law (35 U.S.C. at Sections 200-212) authorizes the licensing of Government-owned patent rights, the FTTA seeks to facilitate technological collaboration at an earlier stage. Thus the FTTA authorizes Federal laboratories to enter into CRADAs, and to agree to grant intellectual property rights in advance to collaborators for inventions made in whole or part by Federal employees under the CRADA. Besides assisting in the transfer of commercially useful technologies from Federal laboratories to the market place, CRADAs make outside resources more accessible to Federal investigators.

The CDC and ATSDR, agencies of the U.S. Public Health Service (PHS), within the Department of Health and Human Services (HHS), are lead Federal agencies for prevention and control of disease and disability in the U. S. and throughout the world. The CDC and ATSDR primary goals are to prevent disease, illness, injuries, and disability before they occur, and to promote health. Under the FTTA, 15 U.S.C. at § 3710(a)2, technology transfer, consistent with mission responsibilities, is also a responsibility of each laboratory science and engineering professional. To achieve its goals, CDC and ATSDR have developed an interdisciplinary research environment and service program that promotes and encourages the free exchange of ideas and information.

In order to safeguard the collegiality and integrity of, as well as public confidence in, the CDC/ATSDR research programs, the following research and technology transfer policies have been adopted.

1. Research Freedom

The CDC and ATSDR investigators generally are free to choose the subject matter of their research, consistent with the mission of their Center/Institute/Office (CIO) and the research programs of their laboratories. No CRADA or license agreement may contravene this freedom.

*Questions or comments about this statement and requests for updated versions should be directed to the CDC Technology Transfer Coordinator at (404) 639-3812.

2. Research Policy

The CDC and ATSDR research results generally are disseminated freely through publication in the scientific literature and presentations at public fora. Brief delays in the dissemination of research results may be permitted under a CRADA as necessary in order to file corresponding patent or other intellectual property applications. The CDC and ATSDR consider the filing of such applications to be an important component of its research efforts.

3. Cooperative Research and Development Under a CRADA

As defined by the FTTA, 15 U.S.C. at § 3710a(d)(1), a CRADA means any agreement between one or more Federal laboratories and one or more non-Federal parties, under which the Government provides personnel, services, facilities, equipment or other resources (but not funds), and the non-Federal parties provide funds, personnel, services, facilities, equipment or other resources toward the conduct of specified research or development efforts. Cooperative research and development activities are intended to facilitate the transfer of federally funded research and development for use by State and local governments, universities and the private sector, particularly small business.

4. CDC and ATSDR CRADAs for Cooperative Research

As adopted by CDC and ATSDR, a CRADA is a standardized agreement intended to provide an appropriate legal framework for, and to expedite the approval of, cooperative research and development projects. The use of CRADAs is encouraged for cooperative efforts because they permit CDC and ATSDR to accept, retain, and use funds, personnel, services, and property from collaborating parties and to provide personnel, services, and property to collaborating parties. The CDC and ATSDR may permit its investigators to enter into CRADAs with collaborators who will make significant intellectual contributions to the research project undertaken or who will contribute essential research materials not otherwise reasonably available. While CDC and ATSDR welcome contributions to its gift funds for research purposes, it does not view CRADAs as a general funding source or a mechanism for sponsored research. This approach to implementing the FTTA has been chosen in order to maintain the public's confidence in CDC and ATSDR through maintaining an independence from reliance on industry funding.

5. Selection of Collaborators under a CRADA

Collaborators under a CRADA may be suggested by potential collaborators or by CDC and ATSDR investigators. Generally, the decision to initiate the approval process for a CRADA is made by the involved CDC or ATSDR investigator and laboratory chief with the approval of Division and CIO directors. Approval is based on scientific considerations and the desire for the public to benefit from the application by the private sector of particular CDC and ATSDR research. For some cooperative projects, where the development and commercialization potential is more immediate relative to the basic research aspects, CDC and ATSDR may seek a collaborator(s) which has both scientific expertise and commercialization capabilities. In certain areas or research, e.g., where the Government has the intellectual lead or where both scientific and commercialization capabilities are deemed essential at the outset, CDC and ATSDR may competitively seek a collaborator(s) through Federal Register notification. The PHS has also developed policy guidelines for ensuring fairness of access to PHS laboratories such as CDC in the process of initiating and developing CRADAs. Additionally, from time to time CDC and ATSDR may sponsor industry collaboration fora to publicize opportunities for collaboration to a wide audience.

6. Proprietary or Confidential Information and Materials

The CDC and ATSDR recognize that an effective collaborative research program may require the disclosure of proprietary information to CDC and ATSDR investigators.

Although agreements to maintain confidentiality are permitted under a CRADA, collaborators should limit their disclosure of proprietary information to the amount necessary to carry out the research plan of the CRADA. The mutual exchange of confidential information, e.g., patient identification data, should be similarly limited. The CDC and ATSDR also recognize that cooperative research may require the exchange of proprietary research materials. Such materials may be used only for the purposes specified in the research plan set forth in the CRADA. All parties to the CRADA will agree to keep CRADA research results confidential until they are published or presented at a scientific meeting.

7. Treatment of Data and Research Products Produced under a CRADA

The CDC and ATSDR investigator and the collaborator will agree to exchange all data and research products developed in the course of research under a CRADA whether developed solely by CDC and ATSDR, jointly with the collaborator, or solely by the collaborator. In general, tangible research products developed under a CRADA will be shared equally by the parties to the CRADA. All parties to a CRADA will be free to utilize such data and research products for their own purposes. Data and research products developed solely by the collaborator may be designated as proprietary by the collaborator when they are wholly separable from the data and research products developed jointly with CDC and ATSDR investigators. However, except as may be afforded through intellectual property rights that require public disclosure of the protected subject matter (e.g., patents), the CDC and ATSDR will not agree to exclude others from utilizing or commercializing the data or research products developed solely by CDC and ATSDR investigators or jointly with the collaborator under a CRADA. Thus, intellectual property which is not patented is not to be afforded trade secret status.

8. Ownership of and Licensing of CDC Intellectual Property Rights

Pursuant to the FTTA, 15 U.S.C. at § 3710a(b)(2), a Federal laboratory is authorized to own and license patent rights to inventions made in whole or part by its employees under a CRADA. The term "invention" is defined at §3703(9) to mean any invention or discovery which is or may be patentable or otherwise protected under Title 35 or any novel variety of plant which is or may be protectable under the Plant Variety Protection Act (PVPA), 7 U.S.C. § 2321 et seq. The patent law, 35 U.S.C. § 207, authorizes the ownership and licensing of intramural inventions. Executive Order 12591 at § 1(b)(1)(B) further authorizes the transfer of Government intellectual property rights. Although the FTTA speaks broadly of the transfer of "technology," CDC and ATSDR do not have statutory authority to license (or to agree to limit dissemination) of technology developed in whole or part by their investigators under a CRADA unless a patent, PVPA certificate or other intellectual property application has been filed for that technology. The CDC and ATSDR will retain the Government ownership interest in, but license rights to, all intellectual property rights to inventions developed solely by their investigators through intramural research or developed in whole or in part under a CRADA.

9. General Licensing Policy

The CDC and ATSDR recognize that under the FTTA and the patent licensing law to which it refers, Congress and the President have chosen to utilize the patent system as the primary mechanism for transferring Government inventions to the private sector. The importance of patents to commercialization in the biomedical field is further reflected by the Drug Price Competition and Patent Term Restoration Act of 1984 (Pub. L. 98-417). A fundamental principle of the patent system is that the owner of a patent has a time-limited "right to exclude others from making, using, or selling the [patented] invention." The reason for such a period of exclusivity is to encourage industry to invest the resources necessary to bring an invention from the discovery stage through subsequent development, clinical trials, regulatory approvals, and ultimately into commercial production. The CDC and ATSDR accordingly are willing

to grant exclusive commercialization licenses under their patent or other intellectual property rights in cases where substantial additional risks, time and costs must be undertaken by a licensee prior to commercialization. Under a CRADA, CDC and ATSDR are also willing to agree to grant exclusive commercialization licenses in advance to collaborators. The CDC and ATSDR will attempt, however, to license their intramural inventions nonexclusively in cases where an invention reflects a relatively more advanced stage in its commercial development, e.g., when a CDC and ATSDR investigator invents a patentable new therapeutic use for a known and FDA approved compound.

Federal laboratories are authorized to negotiate license agreements for Government-owned patent rights in intramural inventions pursuant to 35 U.S.C. § 207 -- Although § 207 does not apply to intellectual property license agreements authorized by the FTTA for inventions made under a CRADA, CDC and ATSDR have adopted the following approach of § 207 for all license agreements:

> *Each Federal Agency [may] . . . grant nonexclusive, exclusive, or partially exclusive licenses under federally owned patent applications, patents, or other forms of protection . . . on such terms and conditions . . . as determined appropriate in the public interest.*

The CDC and ATSDR have determined it to be appropriate and in the public interest to grant nonexclusive research licenses and either exclusive or nonexclusive commercialization licenses to HHS owned intellectual property rights according to the plan discussed below.

10. Government Invention Rights

For inventions developed wholly by CDC and ATSDR investigators or jointly with a collaborator under a CRADA, CDC and ATSDR are required by the FTTA at 15 U.S.C. § 3710a(b)(2) to retain at least a nonexclusive, irrevocable, paid-up license to practice the invention or to have the invention practiced throughout the world by or on behalf of the U.S. Government. When granting exclusive or partially exclusive licenses to CDC and ATSDR intramural inventions, 35 U.S.C. § 208, as implemented by 37 C.F.R. § 404.7(2)(i), requires the reservation of similar Government rights. The CDC and ATSDR will not assert an ownership right in inventions made solely by a collaborator under a CRADA, but will require the grant of a research license, as described below, to the Government for inventions made wholly by collaborator under a CRADA.

11. Research Licenses

The CDC and ATSDR will reserve the right under any CRADA and intellectual property license to grant nonexclusive licenses to make and to use such property, for purposes of research involving the invention itself and not for purposes of commercial manufacture or in lieu of purchase as a commercial product for use in other research. The purpose of the research license is to facilitate basic academic research. The CDC and ATSDR intend to consult with any involved commercialization licensee(s) before granting research licenses to commercial entities. The grant of any research license hereunder does not permit CDC and ATSDR to grant any license to third parties to use the invention for any commercial purpose.

12. Commercialization Licenses

The CDC and ATSDR are willing to consider requests for nonexclusive or exclusive commercialization licenses to intellectual property rights to inventions developed under a CRADA or in the course of intramural research pursuant to applicable statutes and regulations. Under a CRADA, CDC and ATSDR generally will grant a time-limited option to negotiate, in good faith, the terms of a license that fairly reflects the relative contributions of the parties, the risks incurred by the collaborator and the costs of subsequent research and development needed to bring the results of CRADA research to the marketplace. The CDC and ATSDR consider the drafting of a model

invention license to serve as the starting point for license negotiations. It is contemplated further that such a model will reduce negotiations essentially to matters of execution fees royalty rates and minimum annual royalties. Royalty rates will be based on product sales and the rates conventionally granted in the field identified in the CRADA's research plan for inventions with reasonably similar commercial potential. Royalty rates generally will not exceed a rate within the range of 5-8 percent for exclusive commercialization licenses. Contingent royalty schemes based on, e.g., patent issuance or nonissuance, and clauses treating the stacking of royalties or packaging of other inventions developed under the CRADA may be provided. Exclusive licensees will be expected to reimburse CDC and ATSDR for intellectual property related expenses, and may be permitted to offset such reimbursement against future product royalties.

13. Nonexclusive Commercialization Licenses

Unless a request for exclusive commercialization license is made under a CRADA or submitted for an intramural invention, CDC and ATSDR will attempt to license their inventions nonexclusively. Such nonexclusive licenses generally will follow the guidelines of 37 C.F.R. Part 404.

14. Exclusive Commercialization Licenses

The CDC and ATSDR exclusive commercialization requires the submission by a prospective licensee of an acceptable development and commercialization plan as described by 35 U.S.C. § 209(a) and subsequent, periodic reports on utilization of the invention as described by § 209(f)(1). All such plans and reports will be treated in confidence and as privileged from disclosure under the Freedom of Information Act. Modification provisions as described by § 209(f)(2)-(4) may apply. In appropriate cases, CDC and ATSDR may also reserve the right to grant separate exclusive commercialization licenses in various fields of use. The remaining provisions of 35 U.S.C. §§ 200-212 will also apply to licenses to CDC and ATSDR intramural inventions. The CDC and ATSDR also consider the following provisions for exclusive commercialization licenses to be necessary and appropriate in the public interest:(stop)

(i) the exclusive licensee must pledge its reasonable best efforts to commercialize a licensed invention and the development and commercialization plan mentioned above may serve as the measure of such efforts;

(ii) the CDC and ATSDR shall have the right, after notice and opportunity to cure, to terminate or render nonexclusive any license granted: (1) if the licensee is not reasonably engaged in research, development, clinical trials, manufacturing, marketing, sublicensing, or other activities reasonably necessary to the expeditious commercial dissemination of the licensed invention; or (2) when the licensee cannot reasonably satisfy unmet health and safety needs;

(iii) in order to maximize the commercialization of the licensed invention in other fields of use not utilized by the exclusive licensee through ongoing development, manufacturing or sublicensing, CDC and ATSDR reserve the right to require the licensee to grant sublicenses to responsible applicants, on reasonable terms, in such other fields of use unless the licensee can reasonably demonstrate that such a sublicense would be contrary to sound and reasonable business practice and the granting of the sublicense would not materially increase the availability to the public of the licensed invention; and

(iv) exclusive licensees to HHS inventions, whether developed under a CRADA or through intramural research, must agree to not unreasonably deny requests for sublicense or cross license rights from future CRADA collaborators when the possibility of acquiring such derivative rights is necessary in order to permit a proposed cooperative research project with CDC and ATSDR to go forward, and the exclusive licensee has been given a reasonable opportunity to join as a party to the proposed CRADA. The grant of any sublicense or cross license rights

hereunder does not grant future CRADA collaborators a license to use or CDC and ATSDR the right to grant to future CRADA collaborators a license to use any inventions of the instant CRADA for any commercial purpose.

15. Compliance under CRADAs with Other Policies

For research conducted pursuant to a CRADA, collaborators must agree to comply with PHS, CDC and ATSDR policies and guidelines concerning, e.g., human subjects research, the use of research animals, recombinant DNA and other policy statements as may be promulgated from time to time.

16. Pricing

HHS has responsibility for funding basic biomedical research, for funding medical treatment through programs such as medicare and medicaid, for providing direct medical care, and more generally, for protecting the health and safety of the public. Because of these responsibilities, and the public investment in the research that contributes to a product licensed under a CRADA, HHS has a concern that there be a reasonable relationship between the pricing of a licensed product, the public investment in the product, and the health and safety needs of the public.

17. Waivers

The CDC and ATSDR will consider requests to modify any of the foregoing policies in special cases where public health exigencies or commercial situations warrant such a modification. Modifications dealing with business terms such as royalties are not decided by the CDC and ATSDR investigators and should be discussed with the appropriate CDC and ATSDR technology management personnel.

18. Special Consideration and Preference under a CRADA

The CDC and ATSDR will give special consideration to entering into CRADAs with small business firms and consortia involving small business firms; and will give preference to business units located in the United States which agree to manufacture substantially in the United States products which embody inventions developed in the course of research under CRADAs.