OMB No. 0925-0195
Expiration Date 4/30/01

ıd Human Services Service **ɔn Research Program** **Application** *ɪs carefully.*	**Leave blank — for PHS use only.** Type / Activity / Number Review Group / Formerly Council Board *(Month, year)* / Date Received

ceed 56 typewriter spaces)
Home Immunoassay for Cyclosporin

3. PRINCIPAL INVESTIGATOR ☐ New Investigator

hard A.	3b. DEGREE(S) B.S. Ph.D	3c. SOCIAL SECURITY NO. *Provide on Personal Data Page.*
VP for R & D	3e. MAILING ADDRESS *(Street, city, state, zip code)* P.O. Box 58093 Salt Lake City, UT 84158-0093	
, number, and extension)	BITNET/INTERNET Address:	

☐ Full IRB **or** ☐ Expedited Review | 4b. Assurance of compliance no. | 5. VERTEBRATE ANIMALS [X] NO ☐ YES | 5a. If "Yes," IACUC approval date | 5b. Animal welfare assurance no.

rough: 06/30/01

7. COSTS REQUESTED
7a. Direct Costs $ 68,520
7b. Total Costs $ 105,173

ıns and addresses)

9. APPLICANT ORGANIZATION *(Name and address of applicant small business concern)*
Protein Solutions, Inc.
P.O. Box 58093
Salt Lake City, UT 84158-0093

10. ENTITY IDENTIFICATION NUMBER: Fed. Tax # 87-045-1813 | Congressional District: 2

11. SMALL BUSINESS CERTIFICATION
[X] Small Business Concern ☐ Women-owned
☐ Socially and Economically Disadvantaged

ИATION: The information identified on ______ pages ______ of this application that is commercial or financial and to the Government in confidence nation shall be used or disclosed rovided that, if a grant is awarded submission of this application, the or disclose the information herein ion does not limit the Government's ed without restriction from another

TEMENT: If this application does ent permitted to disclose the title ame, address, and telephone num- ant organization, to organizations for further information or possible

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: J. D. Andrade
Title: President and CEO
Address: Protein Solutions, Inc.
P.O. Box 58093
Salt Lake City, UT 84158-0093

Telephone: (801)583-9301
FAX: (801)583-4463
BITNET/INTERNET Address: biolight@concentric.net

ANCE: I certify that the statements o the best of my knowledge. I am it statements or claims may subject ties. I agree to accept responsibility d to provide the required progress this application.

SIGNATURE OF PERSON NAMED IN 3a *(In ink. "Per" signature not acceptable.)* — [signature] — DATE 12/12/99

TIFICATION AND ACCEPTANCE: ue, complete, and accurate to the gation to comply with Public Health awarded as a result of this applica- or fraudulent statements or claims istrative penalties.

SIGNATURE OF PERSON NAMED IN 14 *(In ink. "Per" signature not acceptable.)* — [signature] — DATE 12/12/99

Abstract of Research Plan

NAME, ADDRESS, AND TELEPHONE NUMBER OF APPLICANT ORGANIZATION

Protein Solutions, Inc.
P.O. Box 58093
Salt Lake City, UT 84158-0093 Phone: 801.583.9301

YEAR FIRM FOUNDED	NO. OF EMPLOYEES *(include all affiliates)*
1988	5

TITLE OF APPLICATION

Home Immunoassay for Cyclosporin

KEY PERSONNEL ENGAGED ON PROJECT

NAME	ORGANIZATION	ROLE ON PROJECT
R. Van Wagenen, Ph.D.	Protein Solutions, Inc.	Principal Investigator
Jessica Smith, M.E.	Protein Solutions, Inc.	Research Engineer
D.J. Min, Ph.D.	Protein Solutions, Inc.	Research Scientist
J. D. Andrade, Ph.D.	Protein Solutions, Inc.	Advisor

ABSTRACT OF RESEARCH PLAN: State the application's broad, long-term objectives and specific aims, making reference to the health-relatedness of the project. Describe concisely the research design and methods for achieving these goals and discuss the potential of the research for technological innovation. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded this description, as is, will become public information. ***Therefore, do not include proprietary or confidential information.*** DO NOT EXCEED 200 WORDS.

A complete panel for renal transplant monitoring should include the immunospuppresive drugs Cyclosporine A, FK-506 (Tacrolimus), creatinine (an ongoing NIH Phase I), and urea (part of an anticipated Phase II grant). The assay panel should require less that 100 microliters of blood, be inexpensive, easy to use, and fast (minutes). An increase in the frequency of monitoring can minimize renal transplant failures via early detection of kidney function loss (a manifestation of early rejection). Patient quality of life can be improved and total medical costs can be reduced.

The narrow therapeutic range, toxicity, and variable pharmacokinetics of both Cyclosporine A and Tacrolimus make frequent monitoring essential. Home assays must be fast, frequent inexpensive, and functional; current methods do not meet these requirements. The function of the transplanted organ must also be monitored, meaning frequent creatinine and urea assays for renal transplants. We are developing luminescence enzyme assays for application to renal transplant monitoring in the home, utilizing a hand held luminometer. This proposal addresses the feasibility assessment of an immunoassay for Cyclosporine A (FK-506 will be addressed in Phase II) utilizing a quantitative, immunochromatography assay "viewed" in the luminometer using a backlighting principle.

Provide key words (8 maximum) to identify the research or technology.

Cyclosporine A, Tacrolimus, immunosuppression, therapeutic drug monitoring, immunoassay

Provide a brief summary of the potential commercial applications of the research.

There are 50,000 individuals in the US with renal transplants. Assuming an ideal Cyclosporine A monitoring frequency of once per week at $100 per assay the total cost is $260M per year. Inexpensive weekly assays for Cyclosporine A and Tacrolimus at a cost of $20 per assay would result in a total cost of $52M - a potential cost savings of $208M to the healthcare system. This analysis does not even include the significant reduction in healthcare costs associated with extending the lifetime of kidney transplants.

Principal Investigator *(Last, first, middle):* Van Wagenen, Richard A.

Budget Justification

Using continuation pages if necessary, describe the specific functions of the personnel and consultants. Read the instructions and justify costs accordingly.

Dr. Richard Van Wagenen will serve as Principal Investigator on this project. He has served as PI on other SBIR grants. He is VP for R & D at Protein Solutions and has played a key role in the development of the CCD based multi-channel analyzer for various metabolites. Rick has extensive background in interfacial phenomena, optics and spectroscopy instrumentation. His background in luminescence and device development will address the hardware and materials aspects of the biosensor development. He will also play the major role in planning and coordination of the project. Dr. D.-J. Min, Research Scientist, has had entensive experience in bioluminescent sensors for phenylalanine and lactate. He will assist with the Phase I feasibility studies including simulation and modeling and the optimization of the reactions. Jessica W. Smith, Research Engineer, has a Masters of Engineering in Bioengineering from the University of Utah and will provide techincal support for the project. This will include assistance with modeling and simulation and assistance with the prototype design and in house studies. She is currently a graduate student in the Ph. D. program in Bioengineering. J.D. Andrade is President/CEO. He is not an employee, but serves in a technical consulting function. Financial: Fringe benefits are 28% of salary and indirect costs are 69% of total direct personnel costs. Consulting costs of $2,400 are requested for Dr. John Holman, Jr., M.D., Associate Professor of Surgery and Director of Renal Transplantation at the University of Utah. See Consultants Section for justification of his role. Supplies: Funds are requested for general lab supplies and biochemicals such as proteins, enzymes, buffers, analytical supplies, inorganic chemicals, lab-ware, etc. Travel: Travel funds are requested for one trip to a conference related to clinical chemistry or therapeutic drug monitoring on the east coast. Other: Funds are requested for external clinical laboratory analyses of some samples to be conducted by Associated Regional University Pathologists, Inc. (ARUP), a major clinical chemistry laboratory located near by which serves the western U.S. Funds are also requested for a partial annual membership fee for the Center of Biopolymers at Interfaces (CBI), a state university industry consortium at the University which provides us with extensive access to research resources and interactions with professors and students. See Resources Section below.

Resources

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. (The research to be performed by the applicant small business concern and its collaborators must be in facilities that are available to and under the control of each party for the conduct of each party's portion of the proposed project.) Indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Include laboratory, clinical, animal, computer, and office facilities at the applicant small business concern and any other performance site listed on the FACE PAGE. Identify support services such as secretarial, machine shop, electronics shop, and the extent to which they will be available to the project. Use continuation page(s) if necessary.

Protein Solutins, Inc. (PSI) occupies 1,400 square feet of research, laboratory, and office space located in the Research Park adjacent to the University of Utah. This space includes a chemistry lab, a biology lab, and optics lab, a conference room and two offices. Space and the equipment noted below are adequate to do the work with the proposed addition of the equipment funding requested. Office equipment is standard and adequate to address the administrative aspects of the project, i.e., computers, laser printer, copy and FAX machines, etc. The company is a member of The Center for Bioploymers at Interfaces (CBI) a state-university consortium which is one of the Centers for Excellence in the Utah State System of Higher Education. CBI membership provides a number of key benefits, i.e., (1) fee for service access to many analytical services at the University at very low rates, e.g., the SEM/TEM facility and the Surface Analytical Facility (XPS, SIMS, etc.), (2) access to specialized lab equipment in the departments of Bioengineering (optics lab) and Chemistry (analytical chemistry labs), and (3) access to faculty members who can provide expertise on a consulting basis. The company has licensed technology from the University of Utah in the area of bioluminescence for biosensor development.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Basic laboratory equipment at PSI includes analytical balances, stirrers, hot plates, a fume hood, oven, pH meter, water baths, HEPA filetered laminar air work areas, steam autoclave,a reverse osmosis-filtered water system, light microscopes, Turner Designs luminometer, camera, vacuum system, a gel electrophoresis system, -80 C freezer, UV-Visible spectrophotometer, and lap top computers. Charge coupled device (CCD) array cameras and associated computers and controllers are also available for the recording and quantification of luminescence experiments to supplement the luminometer. A Virtis Genisis 12 EL freeze dryer is available for lyophilization studies of proteins, enzymes and reagents.

Principal Investigator *(Last, first, middle):* Van Wagenen, Richard A.

Biographical Sketch

Provide the following information for the key personnel listed on Page 2, beginning with the Principal Investigator. Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Richard A. Van Wagenen	VP for R & D and Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(If applicable)*	YEAR CONFERRED	FIELD OF STUDY
University of Utah - Salt Lake City, Utah	B.S.	1971	Mechanical Engineering
University of Utah - Salt Lake City, Utah	Ph.D.	1976	Materials Science
University of British Columbia - Vancouver, B.C.	Post Doc.	1976-1977	Cell Surface Research

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, authors, and complete references to those publications most pertinent to this application **DO NOT EXCEED TWO PAGES.**

Professional Experience

- Vice President of Research and Development, Protein Solutions, Inc., Salt Lake City, UT 1996 - present. Current employment.
- RVW Consulting, Salt Lake City, UT 1995-1996.
- Director of Research and Development, OHMEDA Medical Systems, Salt Lake City, UT 1990-1994.
- Vice President Research and Development, Albion Instruments, Salt Lake City, UT 1987-1990.
- Director of Instrumentation, Biomaterials International, Inc., Salt Lake City, UT 1981-1987.
- Research Assistant Professor, Department of Bioengineering, University of UT, Salt Lake City, UT 1978-1985.
- Post Doctoral Fellow, University of British Columbia, Vancouver, B.C. 1976-1977.

Honors

- Member of Pi Tau Sigma, National Honorary Mechanical Engineering fraternity.
- Winner of the NASA Innovation Award, 1981.
- Recipient of the R & D Award for Innovative Design and Development of the RASCAL II; a laser based respiratory/anesthetic gas monitor utilizing Raman light scattering, 1993.

Representative Patents and Publications:

- J.D. Andrade, C.-Y. Wang, D.-J. Min, C. Eu., **R. Van Wagenen,** and R. Scheer, "Toward Dollar Devices for Measuring Metabolic Biochemistry" in *Anti-Microbial, Anti-Infective Materials*, S.P. Sawan and G. Manivannan, eds., Technomic Publishing, 1999, in press.
- D. Gregonis, **R. Van Wagenen**, D. Coleman, and J. Mitchell, "A Commercial Anesthetic Respiratory Gas Monitor Utilizing Raman Spectroscopy", J. SPIE 1336 247 (1990).
- D. R. Westenskow, K.W. Smith, D.L. Coleman, D.E. Gregonis, and **R.A. Van Wagenen**, "Clinical Evaluation of a Raman Scattering Multiple Gas Analyzer for the Operating Room" Anesthesiology, 70 350 (1989).
- **R.A. Van Wagenen**, D.R. Westenskow, R.E. Benner, D.E. Gregonis, and D.L. Coleman, "Dedicated Monitoring of Anesthetic and Respiratory Gases by Raman Scattering", J. Clinical Monitoring, 2 215 (1986).

Principal Investigator *(Last, first, middle):* Van Wagenen, Richard. A.

Biographical Sketch

Provide the following information for the key personnel listed on Page 2, beginning with the Principal Investigator. Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
J. D. Andrade, Jr.	President/CEO and Technical Advisor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
San Jose State University - San Jose, CA	B.S.	1965	Materials Science
University of Denver - Denver, CO	Ph.D.	1969	Materials Science

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronologicabrder, the titles, authors, and complete references to those publications most pertinent to this application**DO NOT EXCEED TWO PAGES.**

Professional Experience

- Professor, Departments of Bioengineering and Materials Science and Engineering, University of Utah 1978 - present. Current employment.
- Founder, President and CEO, Protein Solutions, Inc. Salt Lake City, UT 1988 - present.
- Co-Director, Center for Integrated Science Education, University of Utah, 1991 - present.
- Chairman, Department of Bioengineering, University of Utah, 1978 - 1981 and 1988 - 1991.
- Dean, College of Engineering, University of Utah, 1983 - 1987.
- Co-Chair, Department of Bioengineering, University of Utah, 1998 - present.

Recent Honors

- Surfaces in Biomaterials Foundation Award for Excellence in Surface Science, 1998.
- Governor's Medal for Science and Technology, 1992.
- Distinguished Alumnus Award, San Jose State University, 1987.
- Distinguished Research Award, University of Utah, 1981.
- Fellow, American Institute Medical and Biological Engineering (AIMBE).

Representative Publications:

- **J.D. Andrade**, et al., "Toward Dollar Devices for Measuring Metabolic Biochemistry", in *Anti-microbial, Anti-Infective Materials*, S.P. Sawan and G. Manivannan, eds., Technomic Publishing (1999) in press.
- D.J. Min, **J.D. Andrade**, and R.J. Stewart, "Specific Immobilization of *In vivo* Biotinylated-Bacterial Luciferase and NAD(P)H:FMN Oxidoreductase", Anal.Biochem. (1999) submitted.
- D.J. Min, R.J. Stewart, and **J.D. Andrade**, "Developing a Biosensor for L-Phenylalanine Based on Bacterial Bioluminescence" in *Bioluminescence and Chemiluminescence: Perspectives for the 21st Century*", A. Roda, et al., eds., Wiley (1999) in press.
- D.J. Min, L. Winterton, and **J.D. Andrade**, "Behavior of Model Proteins, Pretreated in Urea and/or Dithiothreitol, at Air/Solution Interfaces", J. Colloid Interface Sci. 197, 43 (1998).
- Q. Luo and **J. D. Andrade**, "Cooperative Adsorption of Proteins onto Hydroxyapatitie", J.Colloid Interface Sci. 200, 104-113 (1998).
- C.-Y. Wang and **J.D. Andrade**, "Surfactants and Coenzyme A as Cooperative Enhancers of the Activity of Firefly Luciferase", in *Bioluminescence and Chemiluminescence: Molecular Reporting with Photons*, W.J. Hastings, et al., eds. Wiley (1997) 253-256.

Principal Investigator *(Last, first, middle):* Van Wagenen, Richard A.

Biographical Sketch

Provide the following information for the key personnel listed on Page 2, beginning with the Principal Investigator. Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Dong-Joon Min	Post Doctoral Staff Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
Chonnam National University, Korea	B.E.	1987	Metallurgical Eng.
Chonnam National University, Korea	M.S.	1989	Metallurgical Eng.
University of Utah, Salt Lake City, Utah	M.S.	1995	Materials Science
University of Utah, Salt Lake City, Utah	Ph.D.	Summer '99	Materials Science

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronologicabrder, the tities, authors, and complete references to those publications most pertinent to this application**DO NOT EXCEED TWO PAGES.**

Research & Professional Experience

- Research Assistant, Department of Chemistry, University of Vermont, 1989 to 1990.
- Research Assistant, Department of Materials Science, University of Utah, 1995 - present.

Honors

- Scholastic Achievement Awards, Chonnam National University, Korea, 1984 - 1987.

Publications

- **D.J. Min** and C.N. Park, "Temperature Dependence of the Chemical Hysteresis in the $LaNi_5$-H System", J. Korean Inst. Metals, 27 836 (1989).
- J.D. Andrade, J. Tobler, M. Lisonbee, and **D. Min**, "Using Bioluminescence for Integrated Science Education", in *Bioluminescence and Chemiluminescence: Status Report*, A. A. Szalay et al. eds., John Wiley, 69-73 (1993).
- J.D. Andrade, M. Lisonbee, and **D. Min**, "Using Novel Biological Phenomena to Enhance Integrated Science Education: Bioluminescence" in *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects*, A.K. Campbell, et al., eds, John Wiley, 371-378 (1994).
- **D.J. Min**, C.Y. Wang, and J.D. Andrade, "Air/Water Monolayer Studies of Bioluminescent Enzymes", *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects*, A.K. Campbell, et al., eds, John Wiley, 494-497, (1994).
- **D.J. Min** and J.D. Andrade, "Preliminary Study of the Optimum Conditions for a Lactate Sensor Based on Bacterial Bioluminescence" in *Bioluminescence and Chemiluminescence: Molecular Reporting with Photons*, W.J. Hastings, et al. eds., John Wiley, 275-278, (1996).
- **D.J. Min**, L. Winterton, and J.D. Andrade, "Behavior of Model Proteins, Pretreated in Urea and/or Dithiothreitol, at Air/Solution Interfaces", J. Colloid Interface Sci. 197, 43 (1998).
- J.D. Andrade, C.-Y. Wang, **D.J. Min**, C. Eu, R. Van Wagenen, and R. Scheer, "Toward Dollar Devices for Measuring Metabolic Biochemistry", in *Anti-Microbial, Anti-Infective Materials*, S.P. Sawan and G. Manivannan, eds., Technomic Publishing (1999) in press.
- **D.J. Min**, R.J. Stewart, and J.D. Andrade, "Developing a Biosensor for L-Phenylalanine Based on Bacterial Bioluminescence", in *Bioluminescence and Chemiluminescence: Perspectives for the 21st Century* , A. Roda, et al. eds., John Wiley (1999) in press.
- **D.J. Min**, J.D. Andrade, and R.J. Stewart, "Specific Immobilization of *In Vivo* Biotinylated Bacterial Luciferase and NAD(P)H:FMN Oxidoreductase" Anal. Biochem. submitted (1999).

Principal Investigator *(Last, first, middle):* Van Wagenen, Richard A.

Biographical Sketch

Provide the following information for the key personnel listed on Page 2, beginning with the Principal Investigator. Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Jessica W. Smith	Research Engineer

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
University of Utah, Salt Lake City, Utah	B.S.	1992	Civil Engineering
University of Utah, Salt Lake City, Utah	Masters	1996	Bioengineering

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, authors, and complete references to those publications most pertinent to this application **DO NOT EXCEED TWO PAGES.**

Research & Professional Experience

- Civil Engineer, Eckhoff, Watson, and Preator Engineering, Salt Lake City, Utah 1991-1995.
- Research Engineer, Orthopedic Biomechanics Institute, Salt Lake City, Utah January, 1996 - February 1997.
- Territory Business Manager, Bristo-Myers Squibb, Salt Lake City, Utah February 1997 - June 1998.
- Research Engineer, Intermountain Health Care, Salt Lake City, Utah June 1998 - present
- Graduate Student, Ph.D. Program, Department of Bioengineering, University of Utah, Salt Lake City, Utah 1998 - present.

Honors

- Professional Engineer License - State of Utah

Publications

- **Smith, J.W.**, Greenwald, A.E., Swanson, S.C., Greenwald, R.M. and Johnson, S.C., "Determination of Patellofemoral Joint Reaction Forces During Low Impact Stepping Exercises", submitted to Medical Science Sports and Exercise, July, 1999.
- Greenwald, R.M., Mecham, M.D., **Smith, J.W.**, Shulimson, A., Nelson, K. and Johnson, S.C., "Neuropsychological Effects of Repeated Head Impacts in Freestyle Aerial Ski Jumping", Abstract #MSSE-2052, presented at ACSM, Denver, CO. 1997.
- **Smith, J.W.**, Motel, R.W., Johnson, S.C., Walker, J.A., Marler, T. and Subudhi, A.W., "The Effect of Concentric and Eccentric Isotonic Resistance Training Programs on Knee Angle Specific Hamstring Quadricep Ratios", Abstract #MSSE-1201, ACSM, Seattle, WA 1999.
- **Smith, J.W.**, Greenwald, A.E., and Greenwald, R.M., " Determination of Patelloformal Joint Reaction Forces as a Function of Cadence During Step Exercises", Abstract #MSSE-218, ACSM Denver, CO 1997.

Principal Investigator (*Last, first, middle*): Van Wagenen, Richard A.

Biographical Sketch

Provide the following information for the key personnel listed on Page 2, beginning with the Principal Investigator. Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
John M. Holman, Jr., M.D., Ph.D.	Consultant & Assoc. Prof. of Surgery

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
Washington and Lee University, Lexington, VA	B.S.	1973	Natural Sciences
U. Texas Southwestern Med. Sch., Dallas, TX	M.D.	1977	Medicine
Albany Medical College, Albany, NY	Ph.D.	1986	Physiology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, authors, and complete references to those publications most pertinent to this application **DO NOT EXCEED TWO PAGES.**

EMPLOYMENT AND EXPERIENCE:

1977-1982, Univ. of Utah Sch. Medicine, Dept. of Surgery, Internship and Residency in General Surgery
1982-1984, Albany Medical College, Dept. of Physiology, NIH Post-doctural Research Fellow
1985-1986, Albany Medical College, Dept of Surgery, Renal Transplant Fellow
1986-1992, Univ. of Utah Sch. Medicine, Assistant Professor of Surgery
1992-present, Univ. of Utah Sch. Medicine, Associate Professor of Surgery
1992, Award of Tenure
1986-1997, University Hospital, Salt Lake City, Assistant Director of Renal Transplantation
1997-present, University Hospital, Salt Lake City, Director of Renal Transplantation

RELEVANT PUBLICATIONS:

Nelson, E.W., Kessler, R., and Holman, J.M., Jr.: Surgical Complications in Pediatric Renal Transplantation. Transplantation Proceedings 21:2006-2007, 1989.

Christensen, A.J., Turner, C.W., Slaughter, J.R., and Holman, J.M., Jr.: Perceived Family Support as a Mediator of Psychological Well-Being in End-Stage Renal Disease. J. Behavioral Medicine 12:249-265, 1989.

Christensen, A.J., Smith, T.W., Turner, C.W., and Holman, J.M., Jr.: Type of Hemodialy-sis and Preference for Behavioral Involvement. Interactive Effects on Adherence in End-Stage Renal Disease. Health Psychology 9:225-236, 1990.

Holman, J.M., Jr., and Todd, R.: Enhanced Survival of Heterotopic Rat Heart Allografts with Portal Venous Drainage. Transplantation 4:229-230, 1990.

Christensen, A.J., Turner, C.W., Smith, T.W., Holman, J.M., and Gregory, M.C.: Health Locus of Control and Depression in End-Stage Renal Disease. J. Consult Clin Psychol 59: 419-424, 1991.

Turner, M.C., and Holman, J.M.: Late Reaction During Initial OKT-3 Treatment. Clinical Transplantation 7:1-4, 1993.

DeVault, G.A., Jr., Kohan, D.E., Nelson, E.W., and Holman, J.M., Jr.: The Effects of Oral Pentoxifylline on the Cytokine Release Syndrome During Inductive OKT3 Transplantation 57(4):532-540, 1994.

THE UNIVERSITY OF UTAH

John M. Holman, Jr., M.D., Ph.D.
General Surgery and
Transplantation Surgery

December 13, 1999

Dr. Rick Van Wagenen
Vice President for R & D
Protein Solutions, Inc.
P.O. Box 58093
Salt Lake City, UT 84158-0093

Dear Rick:

This letter is to confirm my willingness to serve as a Technical Consultant on your Phase I SBIR grant directed towards the development of a Cyclosporin assay system for use in a home healthcare monitoring environment.

In my 15 years as a surgeon specializing in transplantation the availability of cyclosporin has made as great an impact in improving clinical outcomes as any advance to date. However it not an easy drug to use clinically. Dosages have to be continually adjusted to maintain blood levels within tight ranges to avoid complications of underdosing (rejection) and overdosing (renal toxicity). The current assay systems require patients to travel to a central reference laboratory for blood draw or have their blood sample transported there. Both approaches result in considerable hassle for the patient or extended turnaround time for the lab result. A point of care determination of Cyclosporin level would allow for prompt alteration in drug dosing and provide the most optimal opportunity for good clinical outcomes. I am very much in support of this proposal.

Per our discussions, a consulting rate of $100/hour for a total of 24 hours over the course of 12 months should be sufficient. Please find enclosed a copy of my NIH biosketch for your files. Best of luck in your efforts to secure SBIR funding on this very important and interesting project.

Sincerely,

John M Holman Jr

John Holman, Jr., M.D., Ph.D.
Associate Professor of Surgery
Directory of Renal Transplantation

Enclosure

School of Medicine
Department of Surgery
50 North Medical Drive
Salt Lake City, Utah 84132
(801) 581-7508
FAX: (801) 581-6612
e-mail: john.holman@hsc.utah.edu

RESEARCH PLAN

A. Specific Aims

Our long-term objective is to develop a point-of-care and home assay panel and measurement device for renal transplant monitoring. The panel will measure the immunosuppressive drugs cyclosporine A (CsA) and FK-506 (tacrolimus). It will also measure creatinine and urea as markers of kidney function. The measurements are quantitative, using a luminescent detection principle, requiring less than 100µl of blood derived using a micro-lancet from a fingertip. The proposed panel/device can be used during the inpatient period to facilitate faster clinical decision-making and for long-term monitoring after transplantation. Such assays are also useful as research tools with which to understand the pharmacokinetics and pharmacodynamics of CsA and FK-506. The Specific Aims:

1. Model and simulate the proposed and existing assays for CsA and perform computer experiments as a function of assay volume, analyte concentration, protein binding, antibody specificity and binding constants, and related parameters. The goal of the modeling and computer experiment exercises is to identify the key parameter values needed for a successful assay, thereby minimizing the number of laboratory experiments required.

2. Acquire, characterize, and qualify the chemicals and materials needed, including CsA; CsA antibodies; gold, carbon, and dyed latex particles (the contrast producing labels for the immunoassay), electroluminescent (EL) paints and panels (to produce the uniform backlighting through which the immunochromatographic line will be viewed), and related reagents and materials.

3. Become thoroughly familiar with existing and evolving immunoassays for CsA and with HPLC assays (the "gold" standard); this will involve collaboration with and training by local analytical laboratories. Refine and improve the model and simulation activities in Aim #1 to reflect this experience.

4. Develop a particle-contrast immunochromatographic assay (ICA) for CsA which will be compatible with our existing biosensor panel and hand held luminometer and will have the sensitivity needed for small blood samples (less than 100 microliter samples are necessary to insure patient compliance with self-testing in the home).

5. Use the output from Aim 1 and Aim 4 to produce a prototype back-lighted luminescence immunoassay for CsA. Perform preliminary calibrations and verify the prototype assay against established methods for CsA analysis (Aim #3). Evaluate the assay in solution for sensitivity, resolution, and dynamics using CsA standards. Determine if feasibility for a home based, low blood volume assay has been established.

Phase II Specific Aims (tentative):

Improve and enhance reagent deposition, lyophilization, storage, and stability;

Develop a uniform, electroluminescent (EL) light panel on which the ICA will be viewed and the circuitry/power supply to pulse and cycle the EL display (this is needed so the EL does not interfere with the bioluminescence output of the creatinine and urea channels.

Determine cross reactivity towards CsA metabolites; evaluation of potential interferences in blood including the various drugs and therapeutics appropriate to transplant patients;

Develop ICA Assay for FK-506 and/or other newer immunosuppressive agents;

Provide for data collection, processing, and presentation and for enhancement of software/user interface to facilitate individual patient data storage, presentation, and assessment.

Perform limited trial and validation with small patient/surgeon pool and compare with existing assays.

B. Identification and Significance of the Research Opportunity

Transplantation is the major therapy for patients with end-stage organ failure (kidney, liver, heart, lung, and pancreatic β-cell). Renal transplantation alone accounts for more than 65% of the total solid organ transplantation in the United States (1,2). More than 11,000 patients receive renal transplantation annually in the US. There are more than 50,000 individuals with functioning renal transplants in the US (3).

Transplant patients must take medications to suppress the host immune system. For renal transplantation, the patients generally take a cocktail of medications, each blocking a different immune pathway. There are a variety of immunosuppression drug protocols. The majority of the protocols include a steroid and CsA (or FK-506) with or without azathioprine (or mycophenolate mofeti). Newer drugs are now being evaluated.

Each of these medications has a different therapeutic range and side effect profile Therapeutic drug monitoring (TDM) is necessary to individually tailor the medication. Monitoring CsA and FK-506 are mandatory due to their narrow therapeutic ranges, toxicity, and variable pharmacokinetics (4,5). The therapeutic ranges have to be tightly controlled to reduce the risk of either underdosage or toxicity. For renal transplant patients, the generally accepted whole blood therapeutic ranges for CsA and FK-506 are 100-200 μg/l (about 0.1μM) and 3-8 μg/l (about 1 to 10 nM) during the maintenance phase, respectively (6,7,8). Slightly higher doses are recommended during induction phase (first two to four months after transplantation). CsA and FK-506 show a greater than 10-fold variation in whole blood concentration for renal transplant patients on a fixed dose (5,9,10). The variable pharmacokinetics is due to individual patient differences in distribution, absorption, and elimination rates.

CsA is a natural hydrophobic cyclic undecapeptide (F.W.=1203) purified from the fungus Tolypocladium inflatum. FK-506 is a hydrophobic macrolide lactone isolated from the fungus Streptomyces tsukubaensis (F.W.=822). CsA and FK-506 dissolve in methanol, ethanol, polyethylene glycol, and polypropylene glycol, but dissolve poorly in water and hexane. Both CsA and FK-506 are immunophilin-binding drugs. Immunophilins are abundant protein-folding enzymes (proline isomerases). The drugs bind to immunophilins to create an active immunophilin-drug complex. CsA binds to immunophilins called cyclophilins. FK-506 binds to immunophilins called FK binding proteins (FKBP). These complexes inhibit calcineurin, a critical enzyme in the cytokine transcription and T-cell activation pathways (11,12). The inhibition of calcineurin results in the blocking of T-cell activation, thus inhibiting the initiation of the immune response. Although CsA and FK-506 are structurally unrelated, the immunosuppression mechanisms are similar.

Overall renal function must also be regularly monitored. The most useful index of renal function is the clearance of creatinine in blood. Creatinine is a low molecular weight, cyclic nitrogenous end product of metabolism with a normal circulating plasma level of roughly 0.1 mM.

During the in-patient post transplantation period clinical lab tests are performed on a daily basis. An ideal test for either CsA or FK-506 should provide real time diagnostic information by having

a short turn around time (<2hrs), thereby supporting clinical decision-making at minimum cost (14). Current assays for CsA and FK-506 are time consuming and expensive. Average turn around time for current assays is 6-8 hours. The charge for a CsA (and metabolites) assay by HPLC is about $100 (52). Immunosuppressants are measured daily during the 30-day inpatient period; measuring CsA alone will cost $3,000. The following year (3 times/week for the first three months, 3 times/ month for the next nine months) will cost $6,300 for CsA monitoring.

The reduced frequency of monitoring is a compromise between the need for monitoring and the inconvenience and cost of hospital or clinic visits. Rejection can occur late after transplantation. A patient with a transplant can be considered a patient with a chronic disease requiring regular monitoring and management. If renal function and drug levels are monitored more closely (once a week), irreversible transplant damage can be minimized (3).

The availability of a home assay for CsA and FK-506, together with creatinine and urea, will permit long-term renal transplant monitoring in the home environment. Such assays will improve convenience and life quality, and minimize renal transplant failures that now go undetected and untreated due to infrequent monitoring. CsA and FK-506 also need to be monitored on a daily basis when medications are changed due to drug interactions (9).

Assuming the ideal monitoring frequency of CsA and FK-506 is once a week for the 50,000 Americans with renal transplants, there is a need for 2.6 million tests per year (total cost=$260 million at $100 per test). Inexpensive assays will lead to a significant overall cost reduction to the patients and to the health care system. If we assume the proposed assay will cost $20 per test, there is a potential cost reduction of about $200 million per year.

In summary, we propose to develop quantitative point-of care immunoassays for CsA and FK-506. The proposed assay utilizes a particle contrast ICA viewed through an EL panel (back lighting) and recorded by a charge coupled device (CCD) camera-based luminometer. The sensor will require less than 100 microliters of blood sample.

C. Background and Experience

Analytical Background:

Currently, several methods are available for measuring CsA and FK-506 in plasma and whole blood: HPLC, mass spectrometry, and various immunoassays (Table 1) (5,16,17,18,19). CsA and FK-506 are almost exclusively eliminated by the liver. These drugs are predominately bound to red blood cells (>50%) due to the high concentration of cyclophilins and FK binding proteins within the erythrocytes. CsA also binds to cell surfaces since certain CsA binding proteins are expressed on the cell surface. For CsA, about 10% of the drug binds to white blood cells (9,15). CsA is also highly bound to plasma lipoproteins, in particular the high- and low-density lipoproteins (15). FK-506 primarily binds to albumin and α_1-acid glycoprotein (5). Whole blood is the recommended sample for CsA due to a temperature-dependent partitioning effect. CsA concentration tends to be increased in erythrocytes in room temperature assays because the affinity between CsA and cyclophilins increases as the temperature decreases below 37 C. (9,15). Higher signal to noise ratios can be achieved using whole blood sample since most CsA is bound to erythrocytes. FK-506 can be measured in both plasma and whole blood (5), although whole blood is generally preferred. Hematocrit effects are also minimized by using whole blood samples.

Table.1 Commercially available immunoassays for CsA and FK-506. Acronyms: Radioimmunoassay (RIA), Fluorescence Polarization Immunoassay (FPIA), Enzyme-Multiplied Immuno Technique (EMIT), Enzyme Linked Immunosorbent Assay (ELISA), and Microparticle Enzyme Immunoassay (MEIA) (5,15,16,17,18,19)

Cyclosporine A (15,16,17,19)			
Method	Vendor	Web site	Signal Detection
RIA	DiaSorin (Still Water, MN)	www.diasorin.com	Radioactivity of labeled antibody
FPIA	Abbott Labs (North Chicago, IL)	www.abbott.com	Fluorescence polarization of labeled antibody
EMIT (marker: glucose-6-phosphate dehydrogenase	Behring (Deerfield, IL)	www.dadebehring.com	NADH absorption at 340nm/700nm
FK-506 (tacrolimus) (5,18)			
ELISA	DiaSorin (Still Water, MN)	www.diasorin.com	Absorbance at 450/630 nm
EMIA	Abbott Labs (North Chicago, IL)	www.abbott.com	Fluorescence

All the assays listed in Table 1 were designed for centralized clinical laboratories and usually require several pretreatment steps and expensive automated instruments for high throughput. Pretreatment steps include cell lysis by surfactant or organic solvent, CsA (or FK-506) solubilization ,and protein precipitation. RIA has short shelf life and needs careful handling due to its radioactivity. The rest of the assays all require relatively expensive instruments. These commercial assays cannot be easily converted to point-of–care nor to home assays.

To detect molecules such as CsA and FK-506, immunoassay is generally chosen due to its sensitivity and specificity. Today's immunoassays generally use fluorescent or chemiluminescent labels and/or enzyme reactions. Spectrophotometric absorption, fluorescence, and luminescence are the major detection methods used today.

Immunoassays fall into two general categories: heterogeneous and homogeneous. Homogeneous immunoassays require no separation steps (for antibody-bound and unbound components); whereas heterogeneous assays require one or more separation steps. The most commonly used homogeneous immunoassay techniques include EMIT and FPIA (22). EMIT is especially useful in detecting small molecules such as therapeutic drugs and hormones. The basic principle is that the enzymatic activity of the marker enzyme—analyte conjugates is

modulated by the binding of the specific antibodies. The activity is decreased when antibodies bind to the analyte-enzyme conjugates due to binding induced conformational change and/or steric hindrance to the active site posed by antibody. Commonly used marker enzymes in EMIT assays are glucose-6-phosphate dehydrogenase and malate dehydrogenase. An EMIT assay for CsA using glucose-6-phosphate dehydrogenase is commercially available, using spectrophotometric measurement of NADH absorption (Table1).

A wide range of dipstick type assays and kits for drug immunoassay have become available since the early 1980s (24-34). The advances in monoclonal antibodies and the advent and of new and more effective methods for antibody and enzyme immobilization have resulted in increased assay specificity and sensitivity. The development of amplification methods, such as enzyme reactions, dye-containing particles, etc., now permit direct visual detection. Although instrumentless immunoassays had only micromolar sensitivities in the '80s, they have low nanomolar sensitivities today (31,32).

Immunoassays now available for therapeutic drug monitoring incorporate a rich alphabet soup of technologies which form the basis of a rich, confusing, and overlapping patent literature and a highly competitive industry. Current assays range from qualitative direct visual threshold measurements to quantitative, instrument systems involving absorbance, fluorescence, light scattering/turbidity, and chemiluminescence. Our goal is not to reinvent the wheel, but rather to develop a simple assay for immunosuppresive drugs to be used in parallel with our existing bioluminescence assays for creatinine and urea.

Cyclosporine is a large molecule with at least several monoclonal antibodies available (36-38). The cyclosporine monoclonals used in existing immunoassays cross react with some of the drug's metabolites, leading to in increased measure of blood cyclosporine concentration (35,36). This current problem with cyclosporine immunoassay will likely be alleviated as new monoclonal antibodies become available. Some of these new monoclonals will specifically measure the drug metabolites (36,37); other new monoclonals are likely to be even more specific for cyclosporine and related drugs (38) and thereby alleviate metabolite interference in the assay.

In view of our interest in luminescence-based approaches, we examined the available chemi-luminescent immunoassays, which generally use galactosidase as the marker enzyme. A chemiluminescent assay would be compatible with our bioluminescent assays for other analytes (41-46), permitting a complete diagnostic panel for kidney transplant patients. We also built on our earlier experience with flouroimmunoassay (47-49), concluding that it, too, is not compatible with our needs. The more we examined bio and chemiluminescence means to directly assay cyclosporine, and the more we studied particle contrast and immunochromatographic methods, the more we concluded that it would be simpler and far more cost-effective to modify and enhance an established immunochromatographic approach (24-34) for our multichannel luminescence readout needs.

The more than three order of magnitude sensitivity improvement over the last 10 years in immunochromatographic based assay methods (31, 32) makes such technology feasible today for the measurement of cyclosporine and even for the measurement of the lower concentration newer immunosuppresants, including FK-506 (40). Although a dipstick type immunosensor for cyclosporine is not now available, the development of such a sensor is very feasible using today's technologies.

We propose to use a particle enhanced immunochromatographic method for such a sensor (Figure 1). The measurement will result in a line of bound antibody containing the contrast

producing particles. Although this line would be difficult to quantitatively detect by direct visual means, it is easy to detect using the light sensitivity and position measuring characteristics of a CCD array. The immunochromatographic assay we propose is basically a highly enhanced particle contrast method, a hybrid of the methods presented by Zuk (29), Litman (23,24,28) Buechler (30), Birnbaum (31,32), and their coworkers. The difference is that we propose to use a dual antibody method, using the new generation of monoclonal antibodies now available for cyclosporine. We will also use enhanced antibody immobilization methods as well as enhanced chemistries for the binding of the contrast particles to the antibody. We will also utilize modern means of pacifying the particles and the antibodies as well as the surfaces of the assay device (62), minimizing nonspecific binding and the potential for aggregation and agglutination. This assay should easily have nanomolar sensitivity (31,32).

The antibody/contrast particle line will be illuminated by an electroluminescent panel in a backlighting mode (discussed in Figure 2). The antibody line will be designed for maximum sensitivity. The luminometer, therefore, functions as a highly sensitive hand-held densitometer essentially measuring the absorbance of the immunochromatographic channels. The small dimensions of the channels make most effective use of the available drug concentration with very small sample volume requirements.

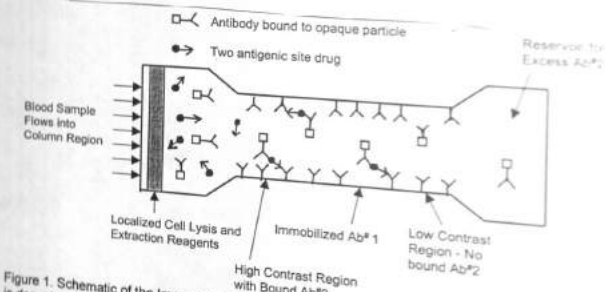

Figure 1. Schematic of the Immunochromatographic Sandwich Drug Sensor. The blood sample is deposited on the left, into a region containing predeposited agents for cell lysis and drug extraction. The particle—labeled specific monoclonal antibody (Ab #2, with the square particle) is also predeposited in the same region. The blood sample hydrates the predeposited reagents and begins to move via capillarity into the chromatographic channel. The drug in the blood is indicated by an arrow, with the head and tail representing the binding regions for each of the two specific monoclonal antibodies. As the cocktail moves into the small channel, it encounters immobilized Ab#1 covalently immobilized on the surfaces of the channel. The drug—Ab#2 complex, pre-formed already in the blood deposition region, binds to the Ab#1 on the walls, producing a bound immuno-sandwich with the contrast producing particle (square) carried by Ab#2. As the lysed blood moves through the channel, the binding events continue until the drug is fully consumed. Excess Ab#2 continues to migrate into the reservoir region at the end of the channel. The result is a line of bound Ab#2 up to the point where binding ceases due to lack of drug. The length of the contrast line is proportional to the drug concentration.

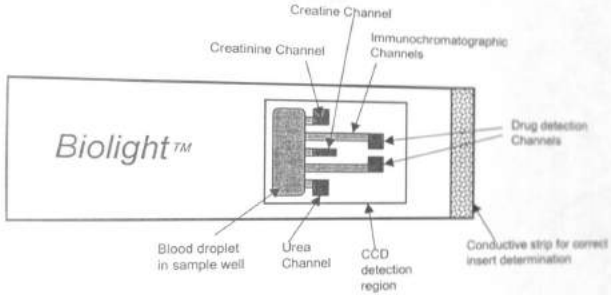

Figure 2. Top View of the biosensor showing the location of the blood sample, the CCD sensing region, and the individual analysis channels. The sensor is held at the left; the right end is inserted into the luminometer.The 50 to 100 microliter blood sample is deposited as shown, constrained to the rectangular geometry by a hydrophobic boundary. The blood penetrates into the device, encounters the predeposited lysing and extraction reagents and begins to move laterally (by capillarity) into the analysis channels, again constrained by hydrophobic barriers and/or discrete channels. In each individual channel, the sample encounters the needed analytical reagents: the upper "well" is for creatinine, the center one for creatine (needed as a reference for the creatinine assay), and the bottom one for urea. Lysed blood also enters the chromatographic channels and binds as noted in Fig. 1, producing a contrast line along the channel. The EL light source (not shown) is in the luminometer, on top of the sensor, shining down through the sensor. The CCD detector (not shown) is under the sensor, receiving the bioluminescence output and the transmitted EL through the drug channels. The EL is off while the CCD is monitoring the metabolite channels and on while monitoring the drug channels. The CCD pixel array (about 600 x 400 elements) readily detects the immunochromatographic line and the length of the line from the origin to where the drug has stopped binding (due to total consumption of the drug). The length of the detected signal is proportional to drug concentration. Such an assay has the sensitivity to detect sub nanomolar cooncentrations in as little as 1-5 microliters of sample.

Relevant Experience

Protein Solutions, Inc. (PSI) is focused on the development and marketing of simple, easy-to-use, inexpensive, and highly quantitative and reliable test strip-type biosensors for use in research settings, point-of-care testing, and for self-monitoring in the home environment. Our long-range goal is the development of the Metabolite Chip or "M" Chip, a multi-channel, luminescence-based analytical device to sense and quantitatively measure up to 100 metabolites. Our experience includes the development of phenylalanine, galactose, and creatinine/creatine luminescence-based diagnostic devices (43-46, 50,51). The development of these sensors has included experience working with several different analytes and enzymes.

It is quite surprising that there are no simple and inexpensive means by which to measure the key metabolites of living systems (43). The only significant exception is the glucose test strip and its companion glucometer which permits the quantitative measurement of glucose in a small drop of blood using reflectance colorimetery or electrochemistry. It is the high incidence of diabetes in the relatively affluent part of the world which has encouraged many companies to invest millions of dollars in the development of simple, inexpensive, high performance analytical instruments focused almost exclusively on glucose.

Our approach for the development of such a device is based on a relatively well-known method; bioluminescence. The bioluminescence in fireflies is based on an enzyme catalyzed oxidation reaction utilizing ATP as a highly specific co-reactant. The bioluminescence of marine bacteria is closely coupled to an NADH-dependent enzyme reaction. The reactions are highly sensitive to and quantitative for ATP or NADH over a five or more order of magnitude concentration range (41). Since all of biochemistry depends on ATP or NADH, practically all metabolic analytes linked to biochemical reactions can be monitored via bioluminescence.

The reactions in each of our enzyme metabolite—specific sensing channels occur simultaneously in a homogeneous or parallel assay sensor. The advantage of a homogeneous assay is that involves fewer assay preparation steps. Homogeneous-type sensors based on bacterial or firefly bioluminescence are of interest for several reasons including; minimal sample handling, ease of use, reliability, and safety.

The sensors are designed for discrete samples. The concentration is measured using a simple disposable device. Both the device and the sample are then discarded. For medical and clinical purposes, the sample of choice is generally blood, usually derived from a simple lancet-based fingertip, earlobe, or heal prick. Modern micro-lancets are almost painless and can readily generate a 100- microliter droplet, adequate for a multi-channel device or panel.

The standard approach to measuring and quantifying bioluminescence generally requires a photomultiplier tube (PMT) based luminometer which can accurately measure intensity. We are developing a CCD camera based luminometer that can quantify the various channels of the M Chip (50). While the sensitivity of the CCD is about 100-1000 times less than a PMT, all of the molecules of metabolism exist in the micromolar to millimolar range which is sufficiently high to be detected by the CCD. The goal is that the instrument will be superior to a PMT based luminometer in that it will be capable of analyzing an inexpensive, disposable multi-channel biosensor card simultaneously for a wide variety of metabolites.

The sensor strip will be inserted into the portable, handheld luminometer. The production version of the instrument will likely be a little larger than a typical home glucometer. Although the initial price will likely be in the $500 range, the final price may be as low as $100 by using photon detection chips based on CMOS technology.

Other multi-channel luminescence based sensors for quantitatively analyzing a spectrum of therapeutic drugs and proteins is our second general goal. It would be highly desirable to monitor the levels of therapeutic drugs that have narrow ranges of effectiveness and toxicity, the classic example being cyclosporin.

Multi-channel biosensors for a family of diagnostically related analytes (metabolites, proteins, drugs, etc.) could be mass produced for a few dollars each. Less than 100 microliters (μL) of blood would be required for operation (0.5 to 2.0 μL of blood plasma per channel) and this could easily be obtained via a painless micro-lancet stick on the finger tip. The patient would not need

to interpret all of the data generated, but would merely download the test results to a remote hospital or clinic data base for storage, additional analysis and review by their clinician. The download could be accomplished with an integral modem or a wireless connection.

Our luminescent approach to analysis has three main components - (1) the biochemistry for analyte specificity and transduction to light that is proportional to analyte concentration, (2) the disposable biosensor which contains the reagents and receives the blood sample, and (3) the analytical device which quantifies the luminescence, stores the result, interfaces to the user and downloads results to a remote patient data base via wireless or modem communications The analytical approach is based on a charge coupled device (CCD) sensor in combination with an optical system that images the array of luminescent biosensor channels onto the CCD. The final luminometer wil;l be battery powered, inexpensive and handheld. CCD based luminometers do exist for high volume clinical chemistry labs, but they are large and expensive.

During the last four years we have developed both the ATP (44,51) and NADH (45,46) biochemistries. Our approach has been based on the following considerations. First, the biotechnology community knows how to express, produce, and purify proteins via simple organism cultures and processes. Indeed, recombinant firefly and bacterial luciferases have been known for some time now and both are commercially available with much more consistency in purity and activity for a reasonable cost. Second, the biotechnology and protein pharmaceutical industries have learned how to formulate, passivate, store, and reconstitute labile proteins and enzymes with considerable retention of activity (53-55). We have addressed the instability of firefly and bacterial luciferase using our experience, understanding, and control of the denaturation of proteins at interfaces and in solution (56). Third, a reaction which produces photons against a dark signal background has the advantage that no light source is required, as is the case with fluorescence, UV-Vis, Raman, and IR spectroscopies. This lowers cost and raises reliability, but more importantly the minimum detection threshold signal to noise (S/N) is improved because there is no noise associated with a large optical background noise. Fourth, one does not require electrodes with their tendencies to become contaminated or to participate in side reactions, as in the case of much of analytical electro-chemsitry.

Background: Preliminary Studies

We have modeled the feasibility of chemiluminescence EMIT assays for CsA and FK-506 by computer simulation. The response of the chemiluminescence immunoassays was simulated using kinetic simulation software KINSIM (58). We showed that the normal therapeutic range of both CsA (100-200 μg/l) and the FK-506 (2-8 μg/l) can be resolved by the proposed assay using a 30 second integration time. The CsA assay starts to show saturation for CsA concentration higher than 240 μg/l. The results suggest that the assays for CsA and FK-506 are feasible in the physiologically relevant concentration range. The resolution and sensitivity of both assays can be further improved by optimizing antibody, drug-β-gal, and chemiluminescence substrate concentrations.

We have used KINSIM, GEPASI (61) and other simulation and theoritical considerations (21) in our modeling activities (57,60).

A quantitative model relating the specified luminous flux of a source to the signal output of the CCD camera was formulated. A valid model is desirable both to confirm that the prototype was functioning as expected and also to develop a predictive method for determining signal levels in future work Radioluminescent photon emission standards were used to determine sensitivity and validate the model.

The requirements of our design call for a low cost, handheld, multi-channel analyzer having a modem or wireless communication capability for networking data to a central medical site. The CCD detector electronics provide for the low noise read-out of the CCD detector, signal amplification, analog to digital conversion, dynamic configuration of the CCD array, automatic detector alignment, data storage, data analysis via implementation of analytical algorithms and serial or parallel communications to the host computer. The device will employ an existing Personal Digital Assistant or PDA such as a 3COM Palm Pilot or a Handspring Visor to provide an inexpensive graphical user interface and serial communications port (Palm Pilot) or parallel communication port (Visor). As the state of the PDA art continues to rapidly advance, it is expected that costs will decrease as performance and capability increase. By building on the efforts of thousands of software developers and utilizing applications and development tools already available, a tremendous advantage can be had in time to market and overall cost of development. Our design philosophy then is to interface standard PDA (Palm Pilot) technology into our handheld analyzer. Our design incorporates custom proprietary electronics to interface to standard PDA technology.

D. Experiments and Methods

Aim 1 – Modeling of the Particle Contrast Luminescent ImmunoAssay (PCLIA):

Enzyme-based biosensors utilize one or more enzyme reactions for specificity and sensitivity. For excellent sensing performance the environment of the biosensor, e.g. buffer type, concentration, pH, and temperature should be controlled for optimal enzyme reaction. Optimal ratios of enzyme amount (or activity) are very important for best sensing performance, including signal output, signal pattern, and resolution. These results can also be changed by assay volume, enzyme status (immobilized or free), and enzyme and reagent concentrations. Modeling (or simulation) is useful to get some information for sensor design, and to most efficiently design experiments (57-61).

Our preliminary models matched experimental results reasonably well (57,59,60). The models can now be extended and used to optimize sensor design.; they allow the designer to adjust enzyme concentrations, ratios, enzyme activity, sample volume, antibody concentrations, binding constants, and specificity to control the nature of the outputted signal. Further, the luminometer model (above) allows the actual output reading to be predicted.

Aim 2 – Biochemicals and Materials

Cyclosporine A is available from Novartis and Sangstat as well as from biochemical reagent suppliers. Monoclonal antibodies against cyclosporine are available from Biodesign International (www.biodesign.com), Advanced Immunochemical, Inc. (www.advimmuno.com), and possibly from Iastechnika (36,37) (www.iastechnika.com). There are also Japanese suppliers. The drug and its antibodies will be obtained and the on and off rates measured using an immobilized antibody method (our paper).

The contrast particles will include dyed latex microspheres, colloidal graphite, and colloidal gold particles. Polysciences, Inc. (*www.polysciences.com*) supplies colloidal gold and colloidal graphite as well as dyed latex spheres. Interfacial Dynamics Corp., Portland, OR supplies a range of dyed latex spheres. The particles will be evaluated with respect to colloid stability, nonspecific antibody binding, and surface attributes for antibody immobilization.

Reagents for passivating the particles and preparing them for antibody immobilization will be obtained from Shearwater Polymers, Inc. (*www.swpolymers.com*) and Pierce Chemical. Reactive polyethylene glycol reagents will be use for antibody immobilization and for particle passivation (62), thus maximizing antibody activity while minimizing nonspecific binding.

Electroluminescent panels will be obtained from Edmund Scientific (*www.edsci.com*) and from other sources. The panels will be characterized with respect to light intensity, homogeneity, and power requirements. Flat capillary channels will be fabricated using glass slide technology, using silicon micro-machining, and using polymer printing and/or stamping (63-65).

Aim 3 – Existing Immunoassays and HPLC Methods for CsA:

We will work closely with colleagues in the Pre-Clinical Drug Evaluation Facility (Dr. Steve Kern) in the Dept. of Pharmaceutics at the University of Utah) in becoming familiar with HPLC methods for CsA and FK506 analysis. We will also work closely with ARUP, Inc. (across the street from us in the University of Utah Research Park), a major clinical chemistry lab (about 100,000 tests per month), in becoming familiar with existing immunoassays for CsA and FK506. We have also budgeted funds for these facilities to provide analysis and testing of our samples.

Aim 4 – Particle Contrast Luminescent Immunoassay:

Our approach here was already described in Figures 1 and 2. Optimum volumes and dimensions will be selected upon the completion of Aims 1 and 3. The modeling will also provide the initial estimates as to antibody concentration (Ab#2), antibody #1 surface concentration, surface concentration of bound Ab#2, etc.

We will continue to evaluate methods for blood lysis and for extraction/debinding of CsA from red cell membrane proteins and other proteins. Such agents are described in the literature for existing assays. For example, conventional assays utilize methanol and zinc sulfate to disintegrate the cell membranes, dissolve CsA (or FK-506), and precipitate the blood proteins. We have a variety of surface treatment methods available to minimize nonspecific binding, absorption, etc. (56, 62).

For hydrophobic barriers, various printing and stamping processes will be evaluated (63-65) which result in high water contact angles at the chamber edges, minimizing fluid migration between chambers. The chamber areas themselves are hydrophilic, coated with PEO copolymers to minimize enzyme absorption and denaturation (62). The mechanical barrier approach utilizes a machined mold; the chamber plastic material is pressed against the mold, resulting in a replica, much as old vinyl records were "pressed" from a master. These methods are now widely employed in microfluidics and micromachining areas (63-65).

The filling of the device and capillary-based migration of the sample is straight forward; displaced air will not be a problem. Some glucose strips have venting holes for air displacement, although that should not be necessary in our design.

The base and top of the sensor is designed to minimize wave-guiding of ambient light into the luminometer and to provide a strip which is easily handled and inserted; only the bottom sensing region is transparent and the portion of the top which is illuminated by the EL source.

The patient produces a lancet generated 50-100 microliter drop of capillary blood, generally from a finger tip. The droplet is touched to the sample application region on the top of the strip

(Fig. 2) and is instantly drawn by capillarity to fully fill the zones. The lysed blood/ "pseudo-plasma" reconstitutes the lyophilized and predeposited analytical reagents in the reaction "chambers" (see caption to Fig. 2)

The strip also contains spacer regions to facilitate its insertion into the luminometer and alinement within the luminometer. The far right end of the strip may also contain an electrically conductive strip to facilitate proper insertion and placement, functioning as a switch which activates the luminometer display to indicate that filling and insertion of the strip has been performed correctly. The strip insertion housing of the luminometer will be removeable for regular cleaning as needed.

We have experience with the immobilization of antibodies and several delicate enzymes, including firefly luciferase (46-49). Although lyophilization and storage stability is mainly a Phase II task, we have used sucrose and trehalose, Dextran 40, and polyethylene glycol (PEG, M.W.=8,000) in our existing preparations.

AIM 5 – Prototype backlighted Immunosensor for CsA:

These studies will use standard assays and methods of analysis, including receiver operating characteristic (ROC) plots and the Clarke Error Grid analysis commonly used to evaluate glucose analysis strips. CsA standards (0, 50, 100, 200, 350, and 500 μg/l) and FK-506 standards (0, 1, 4, 7, 10, and 13 μg/l) prepared in blood will be used to reconstitute the reagents. Different integration windows and integration times will be tested. Interference from blood constituents will be studied. A calibration curve using blood standards will be established. The sensitivity, resolution, dynamic range will be determined. The biosensors will first be tested in simple buffer solutions, followed by more comprehensive testing in commercially available blood certified free of HIV and hepatitis antigens. Internal standards will be used for calibration. The accuracy, linearity and precision of the assays will be determined. Variations between various sensors will also be determined. Our results will be referenced against standard assays via the clinical chemistry services of ARUP, Inc. in Salt Lake City, UT. We will utilize common methods of evaluating analytical performance.

Summary -- We propose to develop point of care chemiluminescence immunoassays for monitoring CsA and FK-506. The complete panel for renal transplant monitoring includes a CsA, an FK-506, and a creatinine (index for renal function) channel. The proposed assays require minimal amount of sample (~50 μl). The assays are inexpensive, easy to use, and fast (turnaround time~ minutes). The assays can also be used as research tools to study the pharmacokinetics and pharmacodynamics of CsA and FK-506.

The proposed assays can be used in the home environment to increase the frequency of monitoring. Renal transplant failures resulting from infrequent monitoring can be minimized; life quality of the patients can be improved; total medical cost can be reduced.

E. Human Subjects: None
F. Vertebrate Animals: None

G. Consultants and Advisors
Our consultant is Dr. John Holman Jr., Associate Professor of Surgery and Surgical Director of Renal Transplantation at the University of Utah. We have budgeted for Dr. Holman's services. Dr. Holman will consult and advise in the design and conduct of the Phase I studies and will

play a major role in the preparation of the Phase II application. His biosketch and letter of participation are enclosed.

No additional consultants are proposed for this development project. We will, however, rely considerably on the expertise of our Scientific Advisory Board members and Dr. Joe Andrade the founder, President, and CEO of Protein Solutions, Inc. Joe has worked extensively with proteins, enzymes, and antibodies for the past 25 years, focusing his efforts on elucidating their behavior at surfaces and interfaces. Joe will be available to assist and consult in the areas of interfacial biochemistry, bioluminescence, and biosensor expertise where required. In addition to Dr. Andrade, our strong Scientific Advisor Board will continue to serve in an advisory and consulting capacity with reimbursement from other sources. The board members are as follows:

Dr. Woody Hastings – Professor of Biology at Harvard University. Dr Hasting is internationally recognized for his basic research on bioluminescence.
Dr. Vladimir Hlady – associate Professor of Bioengineering at the University of Utah. Dr. Hlady is an expert on the study of proteins at surfaces using interfacial fluorescence spectroscopy in conjunction with CCD detectors.
Dr. Henry Kopecek – Professor of pharmaceutics and Bioengineering at the University of Utah. Dr. Kopecek is an internationally recognized authority on hydrogels and related polymers for drug delivery and biocompatibility.
Dr. Larry Kricka – Director of the General Chemistry Lab and Professor of Pathology and Laboratory Medicine at the University of Pennsylvania. Dr. Kricka is internationally recognized for his work on applying both bioluminescence and chemiluminescence to clinical chemistry.
Dr. Russell Stewart – Assistant Professor of Bioengineering at the University of Utah. Dr. Stewart is an expert on recombinant techniques for the synthesis and study of luciferases and motor proteins.

H. Contractural Arrangements—None

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