6b

77-01

SECTION I - RESEARCH PROTOCOL

1) Title — The development of enzyme linked immunosorbent assay (ELISA) techniques for routine and research investigations in the Cholera Research Laboratory

2) Principal Investigator — Dr. Brian Seaton

3) Starting Date — As soon as protocol approved

4) Completion Date — One year after starting date

5) Total Direct Cost — $16,459

6) Abstract Summary

The purpose of this project is to develop a rugged, low-cost instrument for performing enzyme-linked immunosorbent assays (ELISA's) both within the CRL and on field studies. This will involve the design and construction of appropriate instrumentation together with the development of suitable enzyme reactions. The value of this project is (a) it will make the powerful ELISA technique available to researchers both in the CRL and in the field, (b) it will generate within the CRL a body of experience in the design and construction of modern, solid-state electronics scientific equipment.

7) Reviews

a) Research Involving Human Subjects _____

b) Research Committee _____

c) Director _____

d) BMRC _____

e) Controller/Administrator _____

SECTION II RESEARCH PLAN

A. INTRODUCTION

1) Objective.

The primary objective of this project is to design and construct suitable instrumentation and to develop suitable reagent systems to enable enzyme linked immunosorbent assays to be performed on a wide range of entities both within the laboratory environment of the CRL and in the field. A secondary objective is to generate within the CRL a fund of experience in the design and construction of modern solid-state electronics scientific instruments.

2) Background

Since its introduction in the early 60s (1,2), radioimmunoassay (RIA) has proved itself to be an immensley powerful tool for the quantification of a diverse range of compounds of biological interest, particularly those which exert their influence at ultra-low concentrations. Two features of RIA are particularly significant in this respect, (i) the very high specificity which is achievable through the careful control of the way in which antibodies are raised (for example, ref. 3), (ii) the extremely high sensitivity of the system such that nanograms, picograms and, in some cases, even femtograms of substances can be detected and quantifies. This sensitivity of RIAs is due to the ease with which trace amounts of radioisotopes can be quantified.

However, in some cases, the need to use radio-isotopically labelled compounds presents severe problems. These problems are not so apparent in the case of compounds which can be prepared synthetically (eg. steroid hormones) where it is possible, in the course of synthesis, to introduce as many as 6 tritium atoms into the molecule. The resulting labelled compounds are both relatively safe and relatively stable (from an isotopic point of view).

Unfortunately, many of the entities which one would like to be able to quantify by RIA cannot be prepared synthetically (eg they may be proteins or virsus). In these cases the only way of lebelling the compound is to add radioactive iodine (either ^{125}I or ^{131}I) to the molecule. This raises three major problems (i) radioactive iodine is very toxic because it is concentrated in the thyroid where its relatively high-energy emissions cause considerable damage (ii) the isotopes have relatively short half-lives (^{125}I 60 days ^{131}I 8 days) which, in turn, means that fresh batches of labelled compound must be prepared at frequent intervals (increasing both the health-hazzard and the costs) (iii) the process of attaching the labelled iodine necessitates chemical modification of the molecule which, in turn, causes a variable degree of alteration to the immunological properties of the molecule.

It is, therefore, not surprising that much effort has been devoted to finding a technique which eliminates the problems associated with the use of radioactive iodine whilst retaining the advantages of specificity and sensitivity of the original RIA technique. One such method is ELISA. (4,5,6)

In the ELISA technique, labelling is achieved not by the use of radio-isotopes but by the use of enzymes which are chemically coupled to either the antigen or the antibody in such a way that both immunological and enzymatic properties are retained. The amount of antibody or antigen present in an assay system is then readily quantified by measuring the level of enzyme activity. Since, in principle, one molecule of enzyme can catalyse any number of conversions, any degree of sensitivity can be achieved by increasing the incubation time for the enzyme-catalysed reaction though, of course, practical considerations impose limits.

ELISA techniques have already been successfully employed in the quantification of a variety of entities of clinical significance (e.g. 7,8) including some which are directly related to the research interests of the CRL(9).

The advantages of ELISA are:-

i. the enzyme-antibody (or antigen) complex is usually extremely stable

ii. the technique is inherently much simpler to perform than current RIA procedures

iii. it is reputed that the instrumentation and reagent costs for ELISA are substantially less than those for RIA (one of us, BS, has considerable reservations about this!).

The chief "disadvantage" of ELISA at the present is that, being still in its infancy, very little is available commercially both in terms of instrumentation or reagents.

3) Rationale

It is essential that the CRL equip itself to undertake immunoassays (either RIA or ELISA) if research work within the laboratory is not to lag behind progress in the international scientific community. Already, there are several projects within the CRL for which immunoassay techniques are desirable or essential. In view of the problems (mentioned previously) associated with the use of iodine-labelled antigens it would seem appropriate that the CRL should concentrate its attention on ELISA techniques.

Unfortunately, because the tech nique is still in its infancy, ELISA instrumentation is not yet available commercially and even if it were it is unlikely that, at this stage, it would be suited to the environment of Bangladesh.

It is therefore considered desirable that the CRL should undertake the development of its own ELISA instrumentation to meet its own particular needs and in the process, aquire a new expertise in the design and construction of scientific instruments.

B. SPECIFIC AIMS

1) To design, construct and evaluate opto-electronic circuits for the determination of the concentration of antigen or antibody in micro-titre walls based on the ELISA technique. Specific questions will be:

(i) are the opto-electronic circuits stable and reproducible?

(ii) is their response linear with optical density?

(iii) what are the advantages and disadvantages of all solid state electronics systems compared with electro-mechanical systems?

(iv) what is the best way of displaying the data for research applications?

(v) what is the best way of displaying the data for routine applications? applications both in the laboratory and in the field?

(vi) can the instrument be constructed to be rugged and "environment-proof", suitable for use under field conditions?

2) To develop and evaluate reagent systems for use in conjunction with the ELISA instrumentation. Specific questions will be:-

(i) what is the most suitable enzyme and can it be readily linked to a diverse range of antibodies or antigens?

(ii) is the antibody-(or antigen-) enzyme complex stable and under what conditions?

(iii) what is the best substrate for the enzyme?

(iv) what is the best chromagen for quantitatively matching the activity of the enzyme with the optical characteristics of the instrumentation?

(v) is the reagent system stable and can it be packaged for convenience of use in the field?

C. METHODS OF PROCEDURE

1) Simple opto-electronics circuits will be constructed using commercially available semi-conductor devices (including integrated circuits). These circuits will be tested for spectral response, stability, linearity, etc. using standard

solutions calibrated using conventional spectrophotometers. The experience and information gained in these studies will be fed back to improve the circuit design until appropriate characteristics (see section B) are achieved.

2) Concurrently with (1) above, experiments will be conducted with various substrate-enzyme-chromagen systems to evaluate them in terms of the desired characteristics (as set out in section B).

3) Complete opto-electronics/substrate-enzyme-chromogen systems will be evaluated and the instrumentation refined to reach a configuration suited to both the research and routine requirements of the CRL.

4) One (or possibly more) prototype ELISA instruments will be constructed for general use and evaluation under normal working conditions.

5) Data will be obtained and analysed by usual statistical procedures to show (1) the short and long term stability of the system (ii) the sensitivity, precision and accuracy of the instrument (iii) the inter-and intra-assay variability of the system.

D. SIGNIFICANCE

The value of this project to the CRL is three fold:-

1. It will make the powerful ELISA technique available for both research and routine use within the CRL. Such a technique would greatly facilitate projects which have a requirement to detect or quantify low levels of entities of biomedical interest (eg rotavirus, enterotoxins, protein hormones etc) for which RIA techniques are either not available or are costly, hazzardous and cumbersome.

2. It will initiate a fund of experience, within the CRL, in the problems of technical innovation (an important aspect of science in general and biomedical science in particular in an increasingly technological world) thereby taking a step away from total dependence on Western technology.

3. The system developed may well be of considerable practical value to workers in other underdeveloped countries and, possibly, to the world in general.

E. FACILITIES REQUIRED

1) No additional office space is required

2) Laboratory space. 20 running feet, within the biochemistry branch, for 1 yr

3) No hospital resources are required

4) No animal resources are required

5) No logistical support is required

6) Major items of equipment. Access to routine biochemistry instruments (e.g centrifuge, spectrophotometer) and to the facilities of the electronics workshop will be required

7) No specialised requirements are envisaged

F. COLLABORATIVE ARRANGEMENTS

It is envisaged that, during the later stages of this project involving the construction of prototype instruments, there will be some active collaboration with other groups who have experience in the design and construction of biomedical equipment. One such group might be Prof. L.Rossi-Bernardi & Dott. M.Luzzana of the Department of Enzymology, University of Milan together with Sr. I.Raffaeli of Advanced Products SpA, Milan, with whom the Principal Investigator has long-standing links.

An important note on patent protection

It is acknowledged that the CRL is not a commercial or profit-making organization and that, therefore, the pursuit of projects for commercial or financial gain has no part in the role of the CRL.

Notwithstanding this, it would seem appropriate that where a project, undertaken by the CRL for what are deemed to be valid and proper scientific reasons, is also found to be of commercial value, the CRL and its financial sponsors should share the financial reward of its work.

Since development of an ELISA system for the CRL could well result in instrumentation and / or reagent systems with a commercial potential, it is proposed that, during the course of this project, the question of securing appropriate patent protection be kept under review.

bs:amk

BIBLIOGRAPHY

1) Berson & Yallow *

2) Ekins *

3) Abraham, Swerdloff & Tuchinsky. J Clin.Endocr.Metab 1971 32 619

4) Voller Bidwell & Bartlet. In: Manual of Clinical Immunology 1976 Ch.69. Eds.Ross & Friedman

5) Engvall & Perlman J. Immunol. 1972 109 129

6) Editorial in Lancet 1976 ii 406

7) Hermann, Hollingdale Collins & Vinson. P.S.E.B.M. 1977 154 285

8) First International Symposium on Immunoenzyme Techniques, INSERM Symposium No 2, Amsterdam 1976

9) Yolken Kim, Clem et al. In preparation.

* To be advised, references arriving in sea freight

ELISA Project

SECTION III - BUDGET

A DETAILED BUDGET

1) PERSONNEL SERVICES

Name	Position	% Effort No. days	Annual Salary	Project Requirements Taka	Dollars
1) Dr.Brian Seaton	Investigator	10%	$18,907	-	1,891 (1)
2) Dr. David Sack	Investigator	10%	$31 250	-	3,125 (2)
3) Mr. Akbar Ali	Br.Hd.Biochem.	10%	Tk 30 864	3,086	--
4) Mr. M. Sobhari	Br.Hd.Electronics	10%	Tk 42 204	4,220	--
5) To be Appointed	Research Assist.	26 Days	Tk 21,048	2 105	--
			Sub total	9 410	5,015

2) SUPPLIES AND MATERIALS

Electronic components including semi conductors resistors, capacitors, power supplies, light-emitting diades, photodiads, instrument cases etc	-	2,100
Reagents, including enggmes & antisera	-	1,050
Sub-total	-	3,150

3) EQUIPMENT

Digital Muttimeter, Hewlett-Packard Model 3465A with accessories	1 required	-	750
	Sub-total	-	750

	TAKA	DOLLARS
4) PATIENT HOSPITALIZATION		-
5) OUTPATIENT CARE		--
6) CRL TRANSPORT		
Mileage - Dacca 100 miles @ Tk 1.40	140	--
7) TRAVEL & TRANSPORTATION OF PERSONS		
Local Travel	--	--
International Travel	--	--
Transport Dacca-Milan(Italy)return	--	1,100
Per diem expenses 14 days @ $36	--	504
Transport - attendance at meeting	--	2,000
Per diem expenses 14 days @ $36	--	504
Sub-total	--	4,108
8) TRANSPORTATION OF THINGS		
Import of supplies 25% of $2 100	--	525
Import of equipment 25% of $1 050	--	263
Sub-total	--	788
9) RENT COMMUNICATIONS & UTILITIES		
Postage, telephone, cables	1,000	--
Rent (Residence)	15,600	--
Utilities (gas, elec., etc. for residence)	6,000	--
Sub-total	22,600	--

	TAKA	DOLLARS
10) PRINTING & REPRODUCTION		
Special reproduction publications, xerox.	6,000	--
11) OTHER CONTRACTUAL SERVICES		
Maintenance of residence	2,872	--
12) CONSTRUCTION RENOVATIONS, ALTERNATIONS	--	--
Grand Total	Tk 41 032	$13,812
	($2,647)	
		$16,459

Footnote

1) Dr. Seaton's salary is paid by the U.K. Government.

2) Dr. Sack's salary is paid by JHU.

3) It is envisaged that the research assistant appointed will also be employed on the Hormone Assay Project and Trace Elements Project, protocols for which have, or will be submitted.

B BUDGET SUMMARY

Category	Year 1 Taka	Dollars
1) Personnel	9 410	5,015
2) Supplies	- -	3,150
3) Equipment	- -	750
4) Hospitalization	- -	- -
5) Outpatients	- -	- -
6) CRL Transport	140	- -
7) Travel, persons	- -	4,108
8) Transportation things	- -	788
9) Rent communications	22,600	- -
10) Printing/reproduction	6,000	- -
11) Contractual services	2 872	- -
12) Construction	- -	- -
Total	Tk 41,032 +	$13,812
Grand total	$16 459	
Conversion Rate	Tk 15 5 = $1.00	

APPENDIX

Justification for the purchase of a digital multimeter

The construction of the ELISA instrumentation will necessitate extensive testing of electronic circuits both to test and validate the design and constructional aspects of the instrument as well as testing its performance. This will require a multifunction test meter (preferably with digital display giving enhanced precision and sensitivity combined with improved read-out). The CPL does not, at present possess such an instrument

The requested digital multimeter is, therefore, not only essential to this project but will also be a general asset to the CPL electronics workshop.

Attachment 1a

ABSTRACT SUMMARY

Development of ELISA

1) This project does not require a subject population

Items 2 thru' 8 are therefore not applicable.